

Revista Mexicana de Fitopatología ISSN: 0185-3309 mrlegarreta@prodigy.net.mx Sociedad Mexicana de Fitopatología, A.C. México

Bautista Baños, Silvia; Barrera Necha, Laura Leticia; Bravo Luna, Leticia; Bermúdez Torres, Kalina 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, vol. 20, núm. 1, enero-junio, 2002, pp. 8-12 Sociedad Mexicana de Fitopatología, A.C.

Texcoco, México

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# Antifungal Activity of Leaf and Stem Extracts from Various Plant Species on the Incidence of *Colletotrichum gloeosporioides* of Papaya and Mango Fruit After Storage

Silvia Bautista-Baños, Laura Leticia Barrera-Necha, Leticia Bravo-Luna and Kalina Bermúdez-Torres, Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, km 8.5 Carr. Yautepec-Jojutla, San Isidro Yautepec, Morelos, México CP 62731. Correspondence to: sbautis@ipn.mx

(Received: October 11, 2001 Accepted: November 29, 2001)

### Abstract.

Bautista-Baños, S., Barrera-Necha, L.L., Bravo-Luna, L., and Bermudez-Torres, K. 2002. 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 20:8-12. Aqueous extracts of leaves and stems of Achras sapota, Annona reticulata, Bromelia hemisphaerica, Carica papaya, Citrus limon, Chrysophylum cainito, Dyospiros ebenaster, Mangifera indica, Persea americana, Pouteria sapota, Spondias purpurea, and Tamarindus indicus from the state of Morelos, Mexico were tested for their antifungal activity against Colletotrichum gloeosporioides in in vitro and in vivo experiments. Papaya and mango fruit were dipped in extracts, inoculated with C. gloeosporioides, and stored for seven days at room temperature to evaluate percentage infection and severity, solid solubles concentration (SSC), and fruit mass loss. Differences were found between the inhibitory effects in vitro and in vivo. Leaf extracts from C. limon and P. americana totally inhibited growth of C. gloeosporioides in vitro. Leaf extracts of C. papaya completely inhibited postharvest rots of papaya, while leaf and stem extracts of D. ebenaster had an adequate fungicidal effect when applied to mango. Infection severity of papaya ranged from 0 to 50% of the fruit surface, while in mango only 25% of the surface was affected. Overall, infection severity was reduced for both papaya and mango when dipped in extracts. SSC and fruit mass loss varied among plant extracts; however, extracts did not affect fruit quality during storage.

Additional keywords: plant extracts, Carica papaya, Mangifera indica.

Resumen. Se probó la capacidad antifúngica de extractos acuosos de hojas y tallos de Achras sapota, Annona reticulata, Bromelia hemisphaerica, Carica papaya, Citrus limon, Chrysophylum cainito, Dyospiros ebenaster, Mangifera indica, Persea americana, Pouteria sapota, Spondias purpurea y Tamarindus indicus, del estado de Morelos,

México en el control de Colletotrichum gloeosporioides tanto en estudios in vitro como in vivo. Frutos de papaya y mango, se sumergieron en los extractos, se inocularon con C. gloeosporioides y se almacenaron durante siete días a temperatura ambiente. Se evaluó el porcentaje de infección y severidad de la enfermedad, el contenido de sólidos solubles totales (SST) y la pérdida de masa. Se observaron diferencias en el efecto inhibitorio entre los estudios in vitro e in vivo. Los extractos de hojas de C. limon y P. americana inhibieron completamente el desarrollo de C. gloeosporioides in vitro. Los extractos de las hojas de C. papaya inhibieron completamente las pudriciones en fruto de la papaya, mientras que los extractos de las hojas y los tallos de D. ebenaster tuvieron un efecto fungicida adecuado en mango. La severidad de la infección en papaya varió de 0 a 50%, mientras que en mango sólo abarcó el 25% de la superficie de la fruta. En general, la severidad de la infección en papavas y mangos se redujo cuando se lavaron en los extractos. El contenido de SST y la pérdida de masa varió entre los extractos aplicados. Sin embargo los extractos no afectaron la calidad durante el almacenamiento.

Palabras clave adicionales: extractos de plantas, *Carica papaya*, *Mangifera indica*.

There is an increasing demand for papaya (*Carica papaya* L.) and mango (*Mangifera indica* L.) fruit in the National and International markets. However, postharvest rots pose one of the biggest problems to commercialize high quality fruit. Anthracnose caused by *Colletotrichum gloeosporioides* [(Penz.) Penz. and Sacc.] is an important disease of these fruits (Alvarez *et al.*, 1987; Snowdon, 1990). For many years, growers and export industries have relied heavily on synthetic fungicides to control anthracnose. However, there is a need to explore alternatives to reduce this important disease as pathogens develop resistance to fungicides, as evidenced on papayas during the last harvest season (Personnal communication: growers). Also, a continuous loss of chemicals has occurred through regulatory actions. Numerous

studies have demonstrated the fungicidal potential of plant extracts against postharvest fungi. Studies on the inhibitory effects of a diversity of extracts to control fungi such as Botrytis cinerea, Glomerella cingulata, Penicillium expansum, C. gloeosporioides, Phomposis mangiferae, Rhizopus stolonifer, Pestalotia psidii and others, have proved the fungicidal potential of extracts (Bautista et al., 2000; Bommarito et al., 1998; Bong et al., 1997; Mohamed et al., 1996; Pandey et al., 1983; Wilson et al., 1997). In these studies growth of the pathogens were affected at some stage in their development (mycelial growth, sporulation, or conidial germination). The state of Morelos, Mexico is rich in botanical biodiversity. The potential value of plant extracts as fungicides prompted this investigation. The objective of our study was to determine the fungicidal effects of various plant extracts on the growth of C. gloeosporioides in vitro and to determine their activity on papaya and mango fruit and, evaluating some fruit quality parameters after storage.

# MATERIALS AND METHODS

**Plant pathogen.** *C. gloeosporioides* was isolated from infected papaya or mango fruit and identified according to Barnett and Hunter (1972). Each stock culture was maintained on potato-dextrose-agar (PDA).

Plant material. For *in vitro* experiments, leaves and stems of *Achras sapota* L. (sapodilla), *Annona reticulata* L. (custard apple), *Carica papaya* L. (papaya), *Citrus limon* L. (lemon), *Dyospiros ebenaster* Retz. (black sapote), *Mangifera indica* L. (mango), *Persea americana* Mill. (avocado), *Pouteria sapota* (Jacq.) H.E. Moore and Stearn (sapote mamey), *Spondias purpurea* L. (red-mombin), and *Tamarindus indicus* L. (tamarind) were collected in the state of Morelos, Mexico. For *in vivo* experiments, besides the above plant material mentioned, leaves of *Bromelia hemisphaerica* L. ('timbiriche') and leaves and stems of *Chrysophylum cainito* L. (star apple) were also collected. All plant materials were rinsed with distilled water, air-dried, macerated and stored in amber bottles until further use.

**Preparation of extracts.** For *in vitro* or *in vivo* studies, extracts were prepared as follows: Aqueous extracts (2:10 w/v) were left at room temperature (25-28°C) for 24 h and vacuum filtered and sterilized. For *in vitro* studies, extracts were incorporated (10:4 v/v) and mixed with Potato Dextrose Agar (PDA) and then autoclaved (Ahmad and Prasad 1995). The pH of each extract was measured before sterilization (pH meter Orion, Model 420A).

**Parameters evaluated for** *in vitro* **studies**. Mycelial inhibition was expressed as the percentage of inhibition of radial growth relative to the control. Five-mm diameter agar disks of *C. gloeosporioides* were placed on the centre of each agar plate amended with plant extracts and incubated in light for a period of 7-days at 25°C. At the end of incubation period, radial growth of the cultures were measured. Three replicate plates were used for each treatment. To collect conidia for sporulation tests, Petri dishes from each treatment (extract)

were flooded with 10 ml sterile distilled water, the surface was scrapped with a sterile glass rod and the resulting suspension was filtered through cotton wool. Aliquots of 0.5-ml of the conidial suspension were transferred to a Neaubauer haemocytometer. Three Petri dishes were used for each treatment. For germination tests, 0.3-ml aliquots of spore suspension containing 2.1x10<sup>6</sup> spores were placed onto 20-mm diameter disks of agar of PDA amended with each extract tested and incubated for seven hours at 20°C. Germination was recorded once the germ tube could be clearly observed. Spores were then stained with lactophenol acid fucsin. Three replicate dishes were used for each treatment. Both sporulation and percentage germination were determined at 40X magnification.

*In vivo* experiments. Papaya fruit var. Maradol and mango var. Ataulfo were harvested from a commercial orchard at Cuautla, Morelos. Experiments were carried out when papaya and mango fruit were turning from green to yellow.

**Fruit preparation**. Fruit were washed in chlorinated water (200-ml liter<sup>-1</sup>) for 15 min, rinsed with distilled water and air-dried. Fruit were dipped in leaf or stem extracts for 20 min and dried. After treatment, papayas and mangoes were evenly sprayed with a spore suspension of *C. gloeosporioides* (1x10<sup>6</sup>).

**Parameters evaluated for** *in vivo* **experiments.** Individual fruit were used as replicates. For each treatment, a sample of 9 fruit was evaluated for papaya and 10 fruit for mango. Fruit were stored in plastic trays at room temperature (25-28°C) and after a 7 day storage period, percentage infection, severity of infection, soluble solids content (SSC) (Hand refractometer Atago 0-32°), and fruit mass loss were evaluated.

**Statistical analysis**. Treatments were arranged in a completely randomized design. Means separation by Tukey's multiple range test (P = 0.05) were carried out for experiments on leaf or stem extracts for the parameters of growth inhibition, sporulation and conidia germination, weight loss and SSC. Percentage disease was analysed using the Chi square procedure. Disease severity was ranked 1 to 5 where 1 = 0% of surface fruit rotten, 2 = 1-25%, 3 = 26-50%, 4 = 51-75%, and 5 = 76-100%.

# RESULTS

In vitro experiments. Leaf extracts of C. limon and P. americana completely inhibited the in vitro radial growth of C. gloeosporioides while leaf and stem extracts of C. papaya, D. ebenaster, M. indica, P. sapota and S. purpurea totally inhibited sporulation. Leaf extracts of C. limon and T. indicus and stem extracts of M. indica significantly reduced (P = 0.05) conidial germination (Table 1). Sporulation, parameter affected most by extracts, did not occur in extracts with pH values from 4.6 to 6.6, while significantly less mycelial growth (expressed as percentage inhibition) and germination were observed with a range of pH values of 5.3 to 5.6.

*In vivo* experiments. In papaya, percentage infection was significantly different (P = 0.001) only when when fruit was

Table 1. Effect of leaf and stem extracts on *in vitro* development of *Colletotrichum gloeosporioides* after 7 day incubation at 25°C<sup>u</sup>.

		Leave	es	Stems				
Plant	pН	Mycelial	Sporulation <sup>w</sup>	Germination	рН	Mycelial	Sporulation (	Germination
specie	(plant extracts)	inhibition (%)v		(%) <sup>x</sup>	(plant extracts)	inhibition (%)		(%)
Control	5.6	$32.5 a^{y}$	$1.8 \times 10^{5} a$	79 bc	5.6	32.5 ab	$1.8 \times 10^{5} b$	79 a
A. sapota	5.5	37.5 ab	$1.0 \times 10^4  b$	61 abc	5.1	42.5 b	$4.6 \times 10^4 \text{ bc}$	54 bc
A. reticula	ta 5.2	22.5 a	1.2 x 10 <sup>5</sup> a	87 a	5.3	27.5 ab	0 d	53 bc
C. papaya	6.3	27.5 a	0 c	83 a	5.9	35.0 b	0 d	82 a
C. limon	5.3	100 d	_z	42 d	6.0	32.5 ab	4.3 x 10 <sup>5</sup> a	72 ab
D. ebenast	er 5.1	37.5 ab	0 c	63 abc	5.1	35.0 ab	0 d	72 ab
M. indica	5.3	37.5 ab	0 c	62 abc	5.5	25.0 ab	0 d	47 d
P. america	na 5.6	100 d	-	72 bc	4.6	30.0 ab	0 d	32 ab
P. sapota	5.2	52.5 b	0 c	82 a	6.2	12.5 a	0 d	79 a
S. purpure	a 5.3	50.0 bc	0 c	86 a	6.6	37.5 b	0 d	75 ab
T. indicus	4.2	65.0 bc	5.3 x 10 <sup>4</sup> b	45 d	3.9	42.5 b	9.3 x 10 <sup>4</sup> bc	65 abc

<sup>&</sup>lt;sup>u</sup>Average of three observations.

dipped with leaf extracts. No infection was observed in fruit treated with extracts of C. papaya while only 20% infection was observed in fruit treated with leaves of P. sapota. Percentage infection of fruit treated with extracts of leaves of A. sapota and C. cainito was 44% or less, i.e. half of that of untreated fruit (89%) (Table 2). In mango, extracts that significantly (P = 0.001) reduced infection to 10% were those obtained from stems of A. reticulata, D. ebenaster, and T. indicus while stem extracts from C. papaya and C. limon and leaf extracts of D. ebenaster reduced percentage infection to 20% (Table 3). For the treated papaya, infection spread over 0 to 50% of the fruit surface, compared with 75% in the control, while in treated mango only 25% of the fruit surface was infected. SSC and mass loss of papaya and mango varied among extracts. Significant differences (P = 0.05) in SSC and mass loss were observed in fruit treated with extracts obtained from leaves while no differences were found in fruit treated with stem extracts. For both papaya and mango fruit, the lowest SSC was in those fruit dipped in leaf extracts of S. purpurea. Mass loss of papaya fruit ranged from 6.3 to 9.7% (leaf and stem extracts of T. indicus) and 11.5% (leaf extracts of S. purpurea) to 16.8% (stems extracts of D. ebenaster) for mango.

### DISCUSSION

In this study we identified extracts from several plant species that showed adequate antifungal activity on *C. gloeosporioides, in vitro* and *in vivo*. The effect was fungicidal or fungistatic and varied among leaf and stem extracts. The effect of foliar and stem extracts on the development of *C. gloeosporioides* varied for *in vitro* experiments compared to those carried out *in vivo* on papaya and mango fruit. However,

leaf and stem extracts of C. limon had a significant effect on C. gloeosporioides development in vitro and on mango fruit respectively. Previous reports have demonstrated the fungicidal effects of extracts from fruit peel of C. limon (Misra et al., 1988). In this present study, extracts prepared from leaf and stems of papaya reduced anthracnose infection of papaya and mango, while *in vitro* studies extracts of *C. papaya* affected sporulation as well. In other studies, R. stolonifer sporulation and infection development of ciruela fruit did not take place when extract of leaf of C. papaya was applied (Bautista-Baños et al., 2000). The fungicidal activity of this plant on humans mycoses and plant diseases has been reported (Giordani et al., 1996). Among numerous components of papaya fruit it is likely that the alkaloid carpaine would be responsible for this effect (Head and Lauter, 1956). Extracts prepared from leaves and stems of D. ebenaster significantly reduced the percentage infection of mango and stopped sporulation of C. gloeosporioides in vitro. Although this plant species has not been reported with fungicidal properties, various plant organs of different species of the genus Dyospiros (D. piscatoria, D. mespiliformes, D. bateri and D. monbuttensis) have been reported to have a broad range of pharmacological effects on human diseases and insecticidal and termicidal effects (Adeniyi et al., 1996; 2000; Odelola and Okorosobo, 1988). Steroids, triterpenoids, and benzopirones are the main chemical components reported for D. ebenaster (Mallavadhani et al., 1998). In this study, other plant species with promising fungicidal or fungistatic effects were P. americana on in vitro studies, P. sapota, and A. sapota in papaya fruit and A. reticulata, and T. indicus in mango fruit. In this study, the infection percentage of fruit dipped in these extracts was reduced three to four times more compared

<sup>&</sup>lt;sup>v</sup>Micelial inhibition based on inhibition of radial growth compared to control.

wSporulation = number of conidia harvested from single Petri dish (mean of 3 plates).

<sup>&</sup>lt;sup>x</sup>Germination = percentage of conidia germinated after 7 h incubation.

 $<sup>^{</sup>y}$ Means followed by the same letter are not significantly different according to Tukeys's multiple test (P = 0.05).

<sup>&</sup>lt;sup>z</sup>Since mycelial growth did not occur, sporulation did not take place.

Table 2. Effect of leaf and stem extracts on infection percentage and severity, soluble solid content and fruit mass loss of papaya var. Maradol inoculated with *Colletotrichum gloeosporioides* and stored for 7 days at 25-28°C.

	Leaf extracts				Stem extracts			
Plant	Infection	Severity of		Mass loss	Infection S	Severity of	Mass loss	
specie	(%)	infectionw	$SSC^x$	(%)	(%)	infection	SSC	(%)
(	(P = 0.001)				(NS)			
Control	89	4	9.6 abcd <sup>y</sup>	8.9 ab	89	4	9.6 a	8.9 a
A. sapota	33	2	9.9 abc	6.8 b	44	2	9.1 a	8.9 a
A. reticulata	66	2	8.2 bcd	6.3 b	44	2	7.9 a	8.4 ab
B. hemisphaeric	ea 80	2	9.4 abcd	8.3 ab	<b>_</b> z	-	-	-
C. papaya	0	1	10.3 abc	8.8 ab	78	2	9.8 a	7.9 ab
C. limon	78	3	8.1 cd	9.1 ab	44	2	8.5 a	8.2 ab
C. cainito	44	2	9.3 abcd	7.4 ab	44	2	9.2 a	7.1 ab
D. ebenaster	89	3	8.6 bcd	7.3 ab	44	2	9.3 a	7.8 ab
M. indica	100	3	8.6 bcd	7.3 ab	80	3	9.2 a	7.8 ab
P. americana	89	3	9.0 abcd	7.2 ab	56	2	8.4 a	8.0 ab
P. sapota	20	2	9.7 abcd	8.2 ab	89	3	9.3 a	7.7 ab
S. purpurea	78	3	7.7 d	6.6 b	67	3	9.4 a	7.8 ab
T. indicus	50	2	10.7 ab	9.7 a	78	2	7.8 a	6.5 b

<sup>&</sup>quot;Severity of infection: 1 = 0% of surface fruit rotten, 2 = 1-25%, 3 = 26-50% and 4 = 51-75%.

Table 3. Effect of leaf and stem extracts on infection percentage and severity, soluble solid content and fruit mass loss of mango var. Ataulfo inoculated with *Colletotrichum gloeosporioides* and stored for 7 days at 25-28°C.

	Leaf extracts				Stem extracts			
Plant	Infection	Severity of		Mass loss	Infection	Severity of	Mass loss	
specie	(%)	infectionw	$SSC^x$	(%)	(%)	infection	SSC	(%)
(P = 0.001)					(P = 0.01)			
Control	80	4	12.6 cde <sup>y</sup>	12.6 b	80	4	12.6 b	12.6 c
A. sapota	60	2	14.3 abc	15.4 a	60	2	16.5 a	13.2 bc
A. reticulata	90	2	15.6 a	12.6 b	10	2	12.1 b	14.9 abc
B. hemisphaeric	a 80	2	12.3 de	11.8 b	_z	-	-	-
C. papaya	50	2	12.2 de	12.7 ab	20	2	11.6 b	14.7 abc
C. limon	80	2	12.3 de	12.2 b	20	2	11.4 b	13.3 bc
C. cainito	70	2	14.6 ab	13.0 ab	40	2	16.1 b	12.5 c
D. ebenaster	20	2	13.3 bcd	12.6 b	10	2	11.9 a	16.8 a
M. indica	80	2	13.0 bcde	12.4 b	80	2	11.8 a	13.4 bc
P. americana	80	2	14.1 abc	13.3 ab	80	2	11.9 b	13.1 bc
P. sapota	60	2	13.0 bcde	13.3 ab	80	2	12.2 b	13.2 bc
S. purpurea	50	2	11.3 e	11.5 b	80	2	12.7 b	13.4 bc
T. indicus	70	2	12.2 de	13.4 ab	10	2	12.3 b	15.6 ab

<sup>&</sup>quot;Severity of infection: 2 = 1-25% of surface fruit rotten, and 4 = 51-75%.

to the control fruit. Only *P. americana* and *A. reticulata* have been reported with fungicidal and insecticidal properties (Aguilar *et al.*, 1996). Alkadienes and acetogenins of avocado and cherimoya seeds are involved in these bioactive effects

(Adikaram *et al.*, 1992; Gleye *et al.*, 2000). In this study, we also evaluated pH values of the plant extracts tested. According to some published reports, the most appropriate pH values of *Colletotrichum* development are in the range of

 $<sup>^{</sup>x}$ Initial SSC = 7.7.

 $<sup>^{</sup>y}$ Means followed by the same letter are not significantly different according to Tukeys's multiple test (P = 0.05). NS = not significant.

<sup>&</sup>lt;sup>z</sup>Plant material not available.

 $<sup>^{</sup>x}$ Initial SSC = 9.7.

 $<sup>^{</sup>y}$ Means followed by the same letter are not significantly different according to Tukeys's multiple test (P = 0.05).

<sup>&</sup>lt;sup>z</sup>Plant material not available.

5.2 to 6.5 (Mathur et al., 1950; Onkar et al., 1995). Comparing control and treatment results, pH values of extracts apparently did not affect Colletotrichum development. Overall, infection severity was less when fruit were dipped in plant extracts compared with control fruit. Perhaps combining the fungistatic effects of plant extracts with other non-chemical postharvest treatments such as extracts, heat treatments and antagonists might control postharvest anthracnose diseases. In general, fruit quality was not significantly affected when fruit were dipped in extracts. Changes observed in fruit mass loss for both papaya and mango might be more related with the lack of a controlled storage than with the effects of plant extracts. The inhibition of C. gloeosporioides development by some plant extracts indicates the possibility of their use as a postharvest treatment. However, further investigation should be undertaken to determine the effects of this plant material on other important fungi which cause postharvest disease in mango and papaya, and the isolation and identification of active compounds.

**Acknowledgments.** We thank Dr. René Arzuffi for his statistical advice and the National Polytechnic Institute COFAA (CGPI-20010748 for financial support.

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