Available online at www.sciencedirect.com
SCIENCE DIRECT®
Postharvest Biology and Technology 29 (2003) 81–92
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Seasonal evaluation of the postharvest fungicidal activity of powders and extracts of huamuchil (*Pithecellobium dulce*): action against *Botrytris cinerea*, *Penicillium digitatum* and *Rhizopus stolonifer* of strawberry fruit

S. Bautista-Baños [a,c,*], E. García-Domínguez [a], L.L. Barrera-Necha [a], R. Reyes-Chilpa [b], C.L. Wilson [c]

[a] *Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, Carr. Yautepec-Jojutla km. 8.5, San Isidro Yautepec, Morelos 62731, Mexico*
[b] *Instituto de Química, Universidad Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, Mexico*
[c] *USDA, ARS Appalachian Fruit Research Station, 45 Wiltshire Road, Kearneysville, WV 25430, USA*

Received 15 April 2002; accepted 30 November 2002

Abstract

The fungistatic or fungicidal effect of powders, and aqueous and ethanolic extracts of seeds, and monthly harvested leaves of huamuchil (*Pithecellobium dulce*) were evaluated for fungicidal activity, against *Botrytis cinerea*, *Penicillium digitatum*, and *Rhizopus stolonifer*. Fungicidal activity of huamuchil powders and aqueous extracts were also evaluated on strawberry fruit during storage. Preliminary characterization of the active compound responsible for the fungicidal effect was carried out using thin layer chromatography and spectrophotometry. Results indicated that powders had the best fungicidal effect, in both in vitro and in situ studies. Fungistatic or fungicidal properties were associated with the plant organ and harvest month. A correlation between high absorbance values of extracts with the fungicidal or fungistatic effect was not observed. The highest fungistatic or fungicidal effect for both in vitro and in situ studies was recorded from extracts of leaves harvested in months having more stressful environmental conditions; the cold season (October–February) and, the dry, hot season (April and June). Attempts to characterize the active compound suggest that kaempferol may be responsible for the fungicidal effect.

Keywords: *Fragaria* × *ananassa* Duch.; Guaymochil; Tamarind; Kaempferol

1. Introduction

Fungal diseases are one of the major limitations on the storage life of strawberry fruit (*Fragaria* ×

* Corresponding author.
E-mail address: sbautista@afrs.ars.usda.gov (S. Bautista-Baños).

0925-5214/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0925-5214(02)00244-2

ananassa Duch.). *Botrytis cinerea*, *Penicillium digitatum*, and *Rhizopus stolonifer* are the main pathogenic fungi to decay strawberry fruit (Snowdon, 1991; Sommer et al., 1992). Control of these fungi is most commonly achieved by field applications of protective fungicides. However, some of the more effective fungicides have not been registered for use in strawberry, and others have developed resistance problems (Paulus, 1990). Alternatives to control postharvest fungi of fruits such as the use of plant extracts have been explored and less infection was reported for ciruela, mango, and papaya after dipping fruit in plant extracts (Bautista-Baños et al., 2000a, 2002).

Huamuchil (*Pithecellobium dulce* (Roxb.) Benth), native to Mexico, is an evergreen tree about 20 m high. This species typically occurs in lowlands where it has high tolerance to heat and salt and grows well in most soil types, characteristics that account for wide distribution along the coast of Mexico. Huamuchil tree bark is commonly used for fencing and tanning and the pods are prepared for food, while seeds are used for medicinal purposes (Aguilar et al., 1996). Bautista-Baños et al. (2000b) reported that among 20 plant species tested in vitro, aqueous extract and powder from leaves of *P. dulce* had significant fungistatic activity against *Alternaria* sp., *Fusarium oxysporum*, and *Pestalotiopsis* sp. In other studies, postharvest rots of yellow and red mombin (*Spondias purpurea*) caused by *R. stolonifer* was reduced significantly by dipping fruit in *P. dulce* leaf extracts (Bautista-Baños et al., 2000a). Similarly, field studies carried out by Montes et al. (1990) demonstrated the fungicidal effects of ethanolic extracts from leaves of this species against *Uromyces appendiculatus* of beans. Arzuffi et al. (1999) and Llanos et al. (1993) reported toxic effects of powders of *P. dulce* seeds and leaves on larvae of fall armyworm (*Spodoptera frugiperda*).

The present paper describes the in vitro fungicidal or fungistatic effects of powders and aqueous and ethanolic extracts from seeds and leaves of *P. dulce* harvested monthly over a period of 1 year against three important postharvest microorganisms of strawberry. In addition, we determined the absorbance values of aqueous and ethanolic extracts of seeds and leaves and evaluated the control of postharvest rots of strawberry following application of powders or aqueous extracts.

2. Materials and methods

2.1. Plant material

Leaves and seeds were harvested from trees grown at the Biotic Products Development Center in Yautepec, state of Morelos, Mexico. Leaves were harvested monthly over 1 year (January–December) while seeds were harvested in April, (fruit production time). Once harvested, leaves and seed were sorted, discarding damaged or diseased material. Plant material was dipped in a 1% sodium hypochlorite solution, rinsed with distilled water and dried in ambient. Leaves and seeds were macerated with the aid of a blender and a grinder, respectively, and stored in amber bottles until further use.

2.2. Isolation of microorganisms

B. cinerea, *P. digitatum* and *R. stolonifer* were isolated from diseased strawberries. Pure cultures were maintained on potato dextrose agar (PDA). In vitro experiments were carried out using cultures of 14-day-old for *B. cinerea*, 7-day-old for *P. digitatum*, and 4-day-old for *R. stolonifer*.

2.3. Preparation of powders and extracts for in vitro and in situ studies

To evaluate plant powders for in vitro studies, 0.5 g of the macerated material was added to 25 ml of PDA and then autoclaved, to obtain a final concentration of 20 g l^{-1} (w/v) of plant powder in the growth medium (Hernández, 1997). For in situ studies, powders were applied proportionally (w/w) to that applied for in vitro. The final powder concentration applied to each fruit was then 16.6 g kg^{-1} per fruit. For powder applications, fruit were carefully rolled over the powder to ensure that the entire fruit was covered.

Aqueous extracts (2:10 w/v) were left at room temperature (25–28 °C) for 24 h, vacuum filtered, and sterilized. For in vitro studies, extracts were

incorporated (10:40 v/v) and mixed with PDA and then autoclaved (Ahmad and Prasad, 1995; Bautista-Baños et al., 2000b). Ethanolic extracts were prepared according to Reyes-Chilpa et al. (1998): plant material was extracted at room temperature with ethanol (1:5 w/v). The extracts were concentrated in a rotary evaporator and 80 mg of the concentrate was added to 1 ml of ethanol. One milliliter of this solution was poured into PDA (16 ml), after autoclaving 5 ml was added to each Petri plate (60 × 15 mm) to obtain a final concentration of 5 g l^{-1} of ethanolic extract in the growth medium.

Powders and aqueous and ethanolic extracts were tested in vitro while only aqueous extracts and powders were assayed in situ.

2.4. Preparation of fruit

Strawberry fruit were harvested from commercial orchards located in Oacalco, Morelos. Two experiments were carried out: the first was to evaluate aqueous extracts and the second to evaluate powders on the incidence of postharvest rots. For both experiments fruit were at the half colored stage (skin was 50% red colored). Fruit were sorted discarding damaged or diseased fruit. For the first experiment, strawberry fruit were individually cleaned with a soft tissue. For the second experiment, fruit were rapidly washed as previously indicated for plant material.

For the first experiment, fruit were dipped in aqueous extracts of leaves or seeds for 15 min and dried at ambient temperature. For powder treatments, fruit skin was uniformly covered with powder. For both experiments fruit were placed in plastic trays, wrapped in plastic bags but not sealed, and stored for 5 days at 4 °C and 1 day at ambient temperature (25 °C).

2.5. Parameters evaluated for in vitro and in situ studies

For in vitro studies sporulation was measured as previously described (Bautista-Baños et al., 2000b). Mycelial growth was also evaluated and expressed as the percent of inhibition of radial growth relative to the control. Five-mm diameter agar disks of each fungus were placed in the center of Petri plates containing powders or plant extracts. The experiment was terminated when mycelium of control plates reached the edges of the plate. Incubation times were 14 days for *B. cinerea*, at 15 °C, 7 day for *P. digitatum* and 4 days for *R. stolonifer* both incubated at 20 °C. Three replicate dishes were used for each treatment.

For in situ experiments percentage infection and the number of decayed fruit were evaluated. To identify fungi, mycelia were examined once spores were observed, under the microscope. Portions of mycelia were fixed in one drop of lactophenol acid. Identification was according to Barnett and Hunter (1972).

For both experiments, each treatment was applied to two replicates, 40 fruit each for the first experiment and 25 fruit each for the second experiment.

2.6. Characterization of flavonols by spectrophotometry and thin layer chromatography (TLC)

To determine the presence of flavonols absorbing at 346 nm, the ethanolic extracts of the seeds and the monthly harvested leaves of this plant species were analyzed by ultraviolet spectroscopy (UV) (Shimadzu UV-160) and by TLC. Ethanolic extract (1 g l^{-1}) of leaves and seeds was analyzed by UV to determine the absorption at 346 nm.

To screen flavonol compounds of seeds and monthly harvested leaves of *P. dulce*, the aqueous and ethanolic extract of seeds, the monthly harvested leaves and the reference compound kaempferol (important component of *P. dulce* seeds and leaves, maximum absorbance at 346 nm, Southon, 1994; Merck, 1996) were analyzed by TLC over Silica gel 60F_{254} plates (Merck 0.25 mm) with the following elution systems: ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:27). Developed TLC plates were sprayed by 1% methanolic diphenylboric acid–β-ethylamino ester (NP), followed by 5% ethanolic polyethyleneglycol 4000 (PEG) and observed under UV-365 nm light and R_f values were then determined (Wagner et al., 1984).

2.7. Statistical analyses

Treatments were arranged in a completely randomized design. ANOVA, standard deviations (S.D.) and means were calculated for mycelial inhibition and sporulation of the three fungi. Percentage disease was analyzed using the χ^2 procedure. Square root transformation was carried out when data were not normally distributed.

3. Results

The effect of powders and aqueous and ethanolic extracts on mycelial growth and sporulation varied according to the treatment and fungus. *Botrytis* was the most affected by powders and plant extracts.

Except for mycelial inhibition of *R. stolonifer*, the effect of *P. dulce* powders on both mycelial growth and sporulation was significantly different ($P = 0.001$) among treatments (Table 1). For *B. cinerea*, the highest mycelial inhibition was obtained with seed powders (78%), while sporulation of this fungus was most affected by leaf powders harvested in January, November and December. For *P. digitatum* powder prepared from leaves harvested in July had the highest fungistatic effect (58%) compared with the control treatment where no inhibition was observed. Similarly, no effect on mycelial growth was observed for *R. stolonifer*. The least sporulation for *Rhizopus* occurred with leaf powders from April and May.

Aqueous or ethanolic extracts did not have any effect on the mycelial inhibition of *R. stolonifer* while significant differences ($P = 0.001$) were observed for *B. cinerea* and *P. digitatum*. *Botrytis* grown on aqueous extracts of leaves harvested in February and November showed the least mycelial growth (mycelial inhibition = 60 and 57%, respectively) compared with the control treatment, coinciding with inhibition were aqueous extracts exhibited high values of absorbance (2.25 and 2.50 Abs). Other treatments that inhibited the mycelial growth of this fungus more than 50% were ethanolic extracts from seeds and leaves harvested from October to December. There was not a close correlation with high absorbance values. For *P. digitatum*, treatments of aqueous extracts of leaves also harvested in October and November inhibited 60% of the mycelial growth while treatments prepared from aqueous extracts of leaves harvested in March and June reduced growth of *P. digitatum* 52 and 53%, respectively. For all these treatments the absorbance values of the aqueous extracts were among the highest: 2.23–2.50 (Figs. 1 and 2).

For the three fungi, there were also significant differences ($P = 0.001$) with sporulation. Aqueous and ethanolic extracts prepared from leaves and seeds harvested in February affected sporulation of the three test fungi. For *Botrytis*, complete inhibition was also observed from aqueous extracts of leaves harvested in January, November, and December, coinciding with the highest absorbance values (2.3–2.5 Abs). The highest absorbance values of the ethanolic extract, in most cases do not coincide with less sporulation as it is shown with the low or zero sporulation recorded for the three fungi and the low or zero absorbance values from seeds (Figs. 3 and 4).

Results of flavonol determination by TLC of the aqueous and ethanolic extracts of each treatment showed that except for the ethanolic extracts of seed and leaves harvested in July, kaempferol was present in the remainder treatments (Table 2). By contrast this compound was identified only in five of the 13 treatments for the aqueous extracts.

In this study, the main fungi isolated from both experiments with strawberry were *B. cinerea* and *R. stolonifer*. For both experiments *B. cinerea* was more frequent as shown by the high number of infected fruit in the first experiment and the absences of *R. stolonifer* from the second experiment (Table 3). Percentage infection was significantly different ($P = 0.05$ and 0.01) among treatments for fruit treated with powders and extracts, respectively. Of the 13 extracts tested, eight promoted fruit infection of fruit dipped in aqueous extracts. The lowest percentage infection was 27% in fruit dipped in aqueous extracts from leaves harvested in August while in fruit powdered the lowest percentage infection (16%) was for those treated with seeds and leaves from October.

Table 1
Effect of *P. dulce* powders of seeds and monthly harvested leaves on *B. cinerea*, *P. digitatum* and *R. stolonifer* on mycelial inhibition and sporulation after an incubation period

Plant organ	*B. cinerea*		*P. digitatum*		*R. stolonifer*	
	Mycelial inhibition (%) $P = 0.001$[a]	Sporulation (10^4) $P = 0.001$[a]	Mycelial inhibition (%) $P = 0.001$[a]	Sporulation (10^5) $P = 0.001$[a]	Mycelial inhibition (%)	Sporulation (10^5) $P = 0.001$[a]
Seeds	78 (0.1)	1.3 (0.01)	25 (0.1)	21.9 (1.9)	0	10.4 (0.4)
Leaves						
January	40 (0.1)	0	0	17.9 (2.3)	0	6.9 (0.1)
February	60 (0.8)	1.0 (0.1)	45 (0.07)	14.0 (1.0)	0	7.2 (0.1)
March	55 (0.1)	0.9 (0.07)	25 (0.04)	24.4 (1.0)	0	11.3 (0.5)
April	40 (0.8)	1.3 (0.03)	33 (0.06)	24.9 (1.9)	0	3.4 (0.07)
May	33 (0.8)	1.0 (0.01)	20 (0.03)	28.7 (3.8)	0	3.2 (0.1)
June	60 (0.1)	2.4 (0.03)	50 (0.06)	21.4 (1.8)	0	4.2 (0.4)
July	40 (0.5)	1.0 (0.01)	58 (0.05)	18.4 (0.5)	0	7.0 (0.2)
August	53 (0.2)	1.5 (0.01)	43 (0.1)	18.0 (1.0)	0	12.3 (0.2)
September	50 (0.3)	4.5 (0.07)	0	14.7 (0.6)	0	10.7 (0.1)
October	30 (0.1)	2.6 (0.02)	45 (0.03)	10.5 (0.6)	0	13.6 (0.01)
November	53 (0.3)	0	45 (0.05)	11.3 (0.6)	0	8.3 (0.1)
December	50 (0.6)	0	33 (0.07)	15.8 (0.7)	0	7.4 (0.2)
Control	20 (0.1)	5.6 (0.02)	0	38.7 (1.5)	0	15.7 (0.08)

Values in parenthesis indicate S.D. of the mean.
[a] P values after square root transformation.

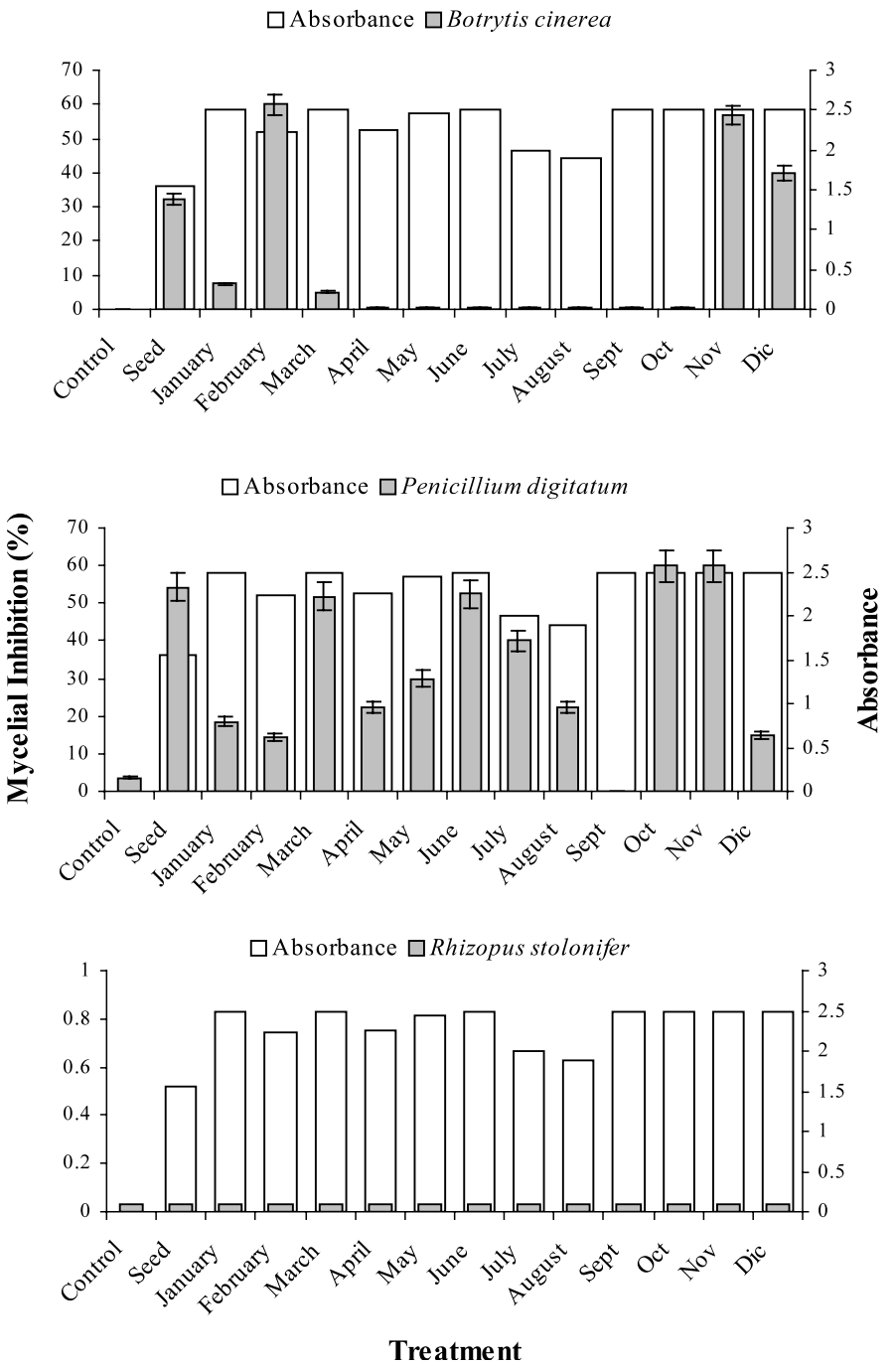

Fig. 1. Relationship of the absorbance of aqueous extracts of *P. dulce* (seeds and monthly harvested leaves) with the mycelial inhibition of *B. cinerea*, *P. digitatum* and *R. stolonifer*. Vertical bars indicate S.D. of the mean.

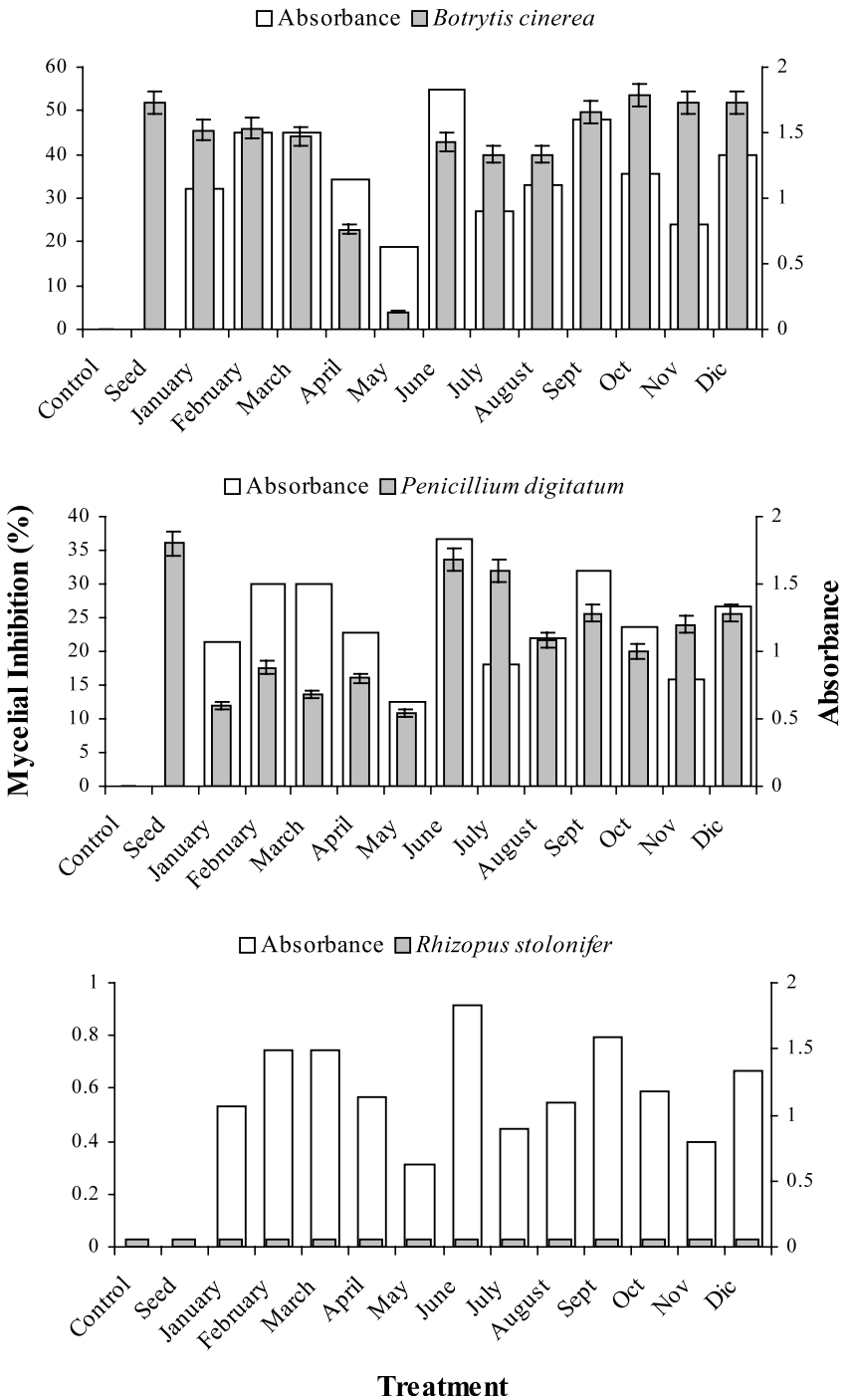

Fig. 2. Relationship of the absorbance of ethanolic extracts of *P. dulce* (seeds and monthly harvested leaves) with the mycelial inhibition of *B. cinerea*, *P. digitatum* and *R. stolonifer*. Vertical bars indicate S.D. of the mean.

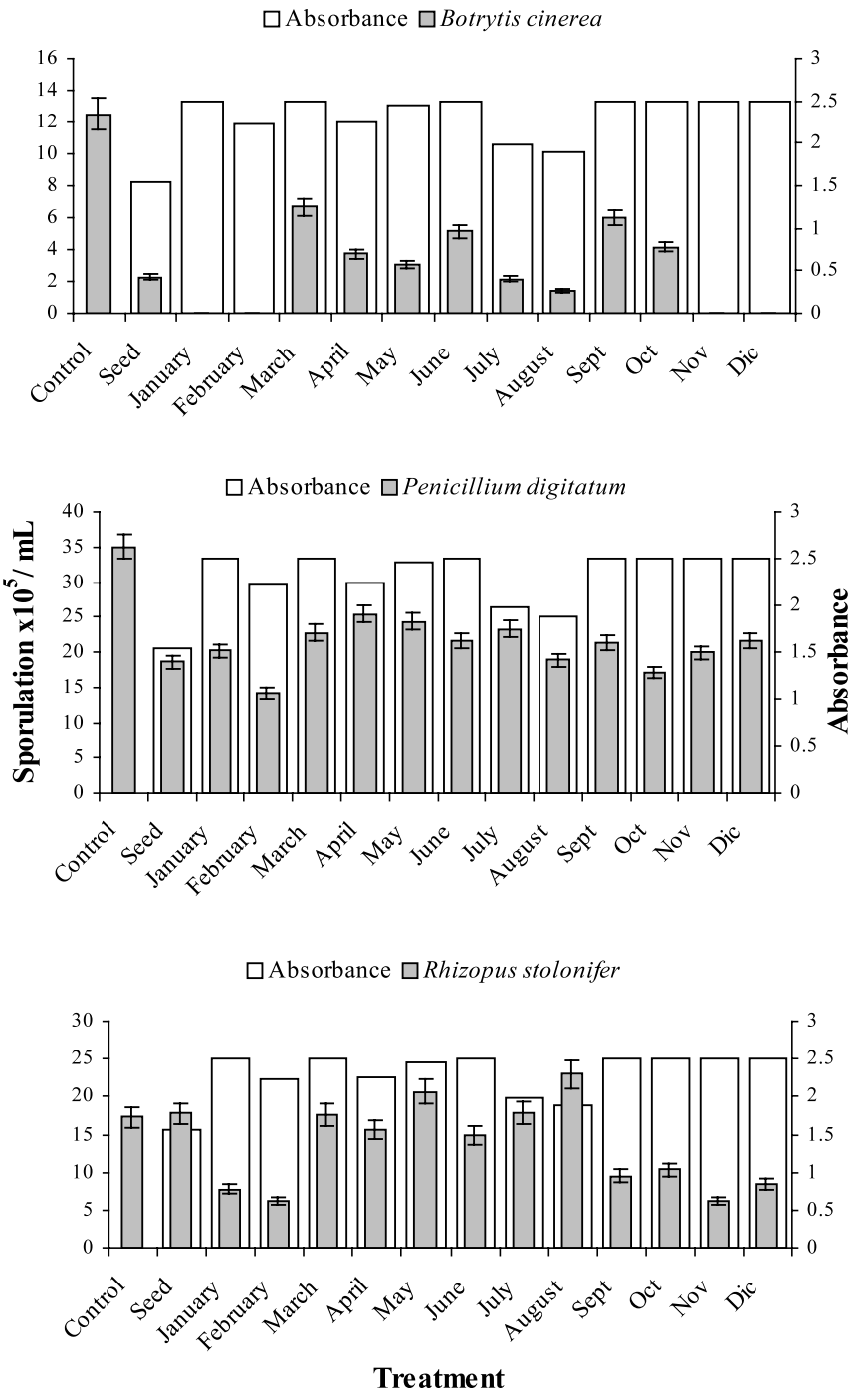

Fig. 3. Relationship of the absorbance of aqueous extracts of *P. dulce* (seeds and monthly harvested leaves) with sporulation of *B. cinerea*, *P. digitatum* and *R. stolonifer*. Vertical bars indicate S.D. of the mean.

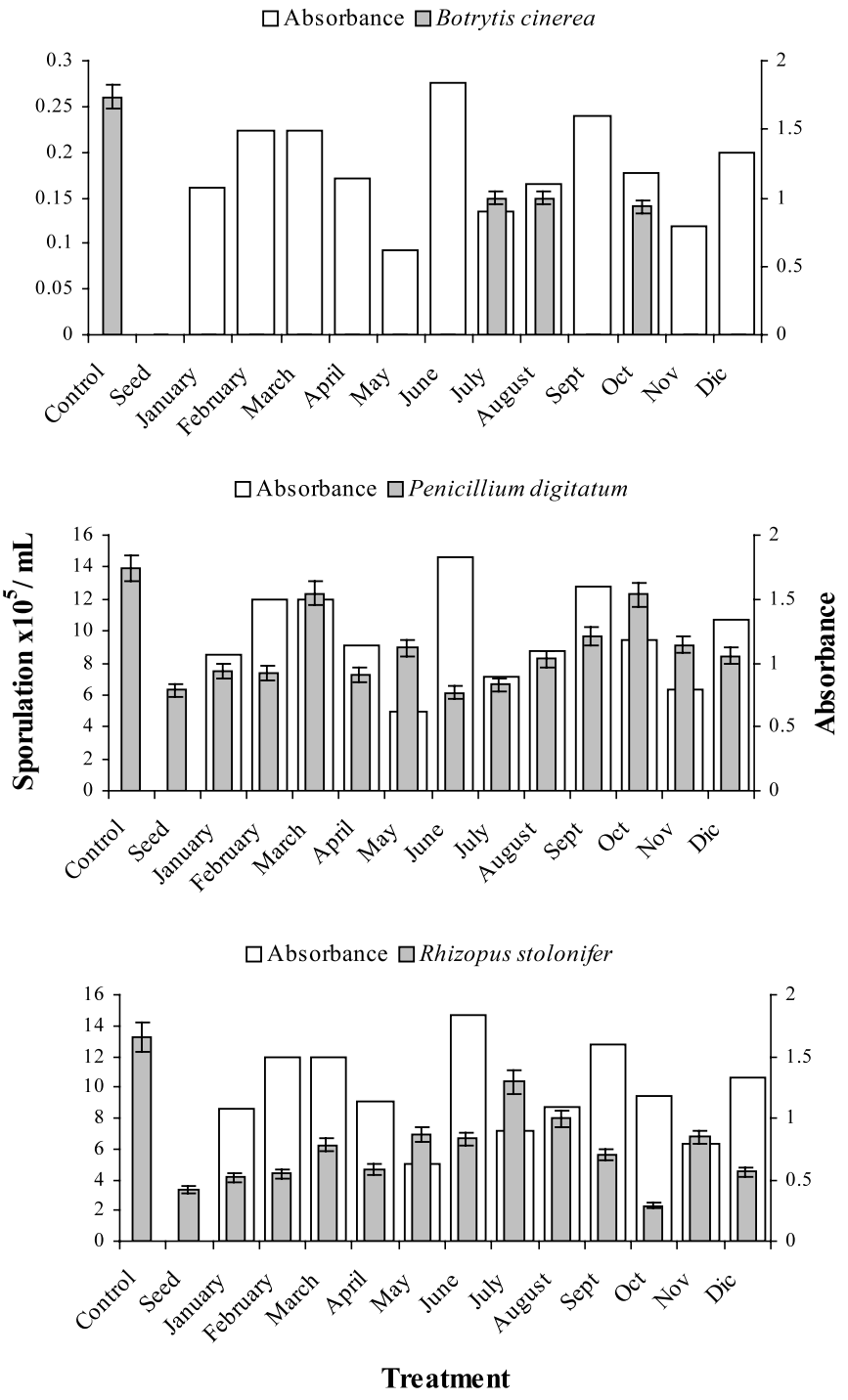

Fig. 4. Relationship of the absorbance of ethanolic extracts of *P. dulce* (seeds and monthly harvested leaves) with sporulation of *B. cinerea*, *P. digitatum* and *R. stolonifer*. Vertical bars indicate S.D. of the mean.

Table 2
R_f and fluorescence of flavonols (kaempferol) in aqueous and ethanolic extracts of *P. dulce* seeds and leaves harvested monthly by thin layer chromatography

Plant organ	Extract			
	Aqueous		Ethanolic	
	R_f	UV (365 nm)	R_f	UV (365 nm)
Seed	–	–	–	–
Leaves				
January	0.95	Yellow	0.91	Yellow
February	0.95	Yellow	0.91	Yellow
March	–	–	0.93	Yellow
April	0.95	Yellow	0.93	Yellow
May	–	–	0.93	Yellow
June	–	–	0.91	Yellow
July	–	–	–	–
August	–	–	0.90	Yellow
September	0.75	Yellow	0.90	Yellow
October	0.75	Yellow	0.90	Yellow
November	–	–	0.90	Yellow
December	–	–	0.90	Yellow
Kaempferol	0.91	Yellow–green	0.90	Yellow–green

–, Zero values.

4. Discussion

Overall, we observed that the degree of fungicidal or fungistatic activity was not constant during the sampling season. In general, *Botrytis* development during in vitro and in situ experiments was more affected by extracts from leaves harvested in cold, dry and also hot months. Aqueous extracts and powders from leaves harvested from October to February inhibited the mycelial growth and sporulation of this fungus in in vitro studies while powders from October harvested leaves also reduced fungal development of strawberry (coolest season). Similarly, leaves harvested in April and June (driest and hottest season) inhibited mycelial development and fruit infection of strawberry. Others have reported the fungicidal effect according to a specific geographical area or active compound. Lira-Saldívar et al. (2001) found differences in the fungicidal effect of leaf extracts of *Larrea tridentata* against *F. oxysporum*, harvested in two different areas of the dessert of Chihuahua and Sonora, Mexico. In that study the

Table 3
Effect of aqueous extracts and powders of seeds and monthly harvested leaves of *P. dulce* on percentage infection and number of decayed fruit by *B. cinerea* and *R. stolonifer* on strawberry fruit

Plant organ	Aqueous extracts			Powders	
	Percentage infection $P = 0.05$[a]	Number of decayed fruit		Percentage infection $P = 0.01$[b]	Number of decayed fruit
		B. cinerea	*R. stolonifer*		*B. cinerea*
Seeds	85	32	5	16	4
Leaves					
January	50	18	3	26	7
February	55	12	10	36	9
March	84	31	3	24	6
April	31	13.0	5	18	5
May	48	12	8	24	6
June	47	19	0	18	5
July	43	17	0	18	5
August	27	11	0	24	6
September	58	21	4	30	8
October	57	15	13	16	4
November	63	9	16	32	8
December	54	15	4	18	5
Control	48	15	14	34	9

[a] Percentage infection based on two replications of 40 fruit each.
[b] Percentage infection based on two replications of 25 fruit each.

highest concentration of resins with antifungal properties was found in those leaves harvested from the Sonoran dessert. The authors stated that these differences might be due to the different latitude and altitude between the Sonoran and the Chihuahuan dessert. Espinosa-García and Langenheim (1991a,b) reported that the fungus *Pestalotia subcutilaris* was differentially affected according to the presence or absence of a particular active compound present in the tree named *Hymenea* spp. Espinosa-García (2001), highlights the significance of identifying the chemical profile of the secondary metabolites of those plants with pesticidal and fungicidal effects. The fungicidal or fungistatic effects of the monthly samples tested in this present research, might be related to changes in environmental conditions i.e. temperature and relative humidity, hence differences with the presence and quantity of specific active compounds with fungicidal activity.

In this study, we did not observe a defined relationship between the high absorbance values of seeds and the monthly harvested leaves with fungicidal or fungistatic effects. However, we did observe a relationship between the inhibition of mycelial growth and less sporulation with high values of absorbance. According to the literature, the yellow–green fluoresces zone at UV 365 nm is likely to be the kaempferol compound (Wagner et al., 1984). The obtained R_f values in this study suggest the presence of this compound. However, an R_f at 0.75 may indicate that the kaempferol is bonded to other compounds such as glycosides. Secondary metabolites are a mixture of different active compounds, each of them acting according to concentration and structure. In this present study, powder application on strawberry fruit compared with aqueous extracts had a better fungicidal effect, since powders were not subjected to an extraction process, they had various and different active compounds that might be acting all together. However, other evaluations carried out in our laboratory have demonstrated the fungicidal effects of the isolated compound kaempferol against various postharvest fungi (data not shown). This compound has been also reported with fungicidal activity against *F. oxysporum* on carnation (Curir et al., 2001).

To date, chemical analyses of leaves and seeds of *P. dulce* have led to the identification of other saponines and flavonols such as quercitin (Nigam and Mitra, 1970; Sahu and Mahato, 1994; Nigam and Gopal, 1997). Therefore, the elucidation of other chemical structures with bioactive potential in seeds and leaves of this plant species against postharvest decay microorganisms of fruit need to be investigated.

Acknowledgements

To the National Polytechnic Institute (Grant: COFAA-CGPI-20010748).

References

Ahmad, S.K., Prasad, J.S., 1995. Efficacy of foliar extracts against pre- and post-harvest diseases of sponge-gourde fruits. Lett. Appl. Microbiol. 21, 373–375.

Aguilar, A., Camacho, J.R., Chino, S., Jacquez, P., López, M.E., 1996. Plantas Medicinales del Herbario IMSS. p. 63.

Arzuffi, B.R., Aldana, L.L.L., Valdés, E. Ma. E., Martínez, M.L., 1999. Efectos tóxicos de *Pithecellobium dulce* sobre gusano cogollero (*Spodoptera frugiperda*). In: IV International Meeting on Sustainable agriculture, 24–27 October, Morelia, Mexico (Abstr. 85).

Barnett, H.L., Hunter, B.B., 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing Co, USA, p. 241.

Bautista-Baños, S., Hernández-López, M., Díaz-Pérez, J.C., Cano-Ochoa, C.F., 2000a. Evaluation of plant extracts to reduce *Rhizopus stolonifer* of 'ciruela' (*Spondias purpurea* L.) fruit during storage. Postharvest Biol. Technol. 20, 99–106.

Bautista-Baños, B.S., Hernández-López, M., Barrera-Necha, L.L., 2000b. Antifungal screening of plants of the state of Morelos, Mexico against four fungal postharvest pathogens of fruits and vegetables. Mex. J. Phytopathol. 18, 36–41.

Bautista-Baños, S., Barrera-Necha, L.L., Bravo-Luna, L., Bermúdez-Torres, K., 2002. Antifungal activity of leaf and stem extracts from various plant species on the incidence of *Colletotrichum gloeosporioiodes* of papaya and mango fruit after storage. Mex. J. Phytopathol. 20, 8–12.

Curir, P., Dolci, M., Lanzotti, V., Taglialatela-Scafati, O., 2001. Kaempferide triglycoside: a possible factor of resistance of carnation (*Dianthus caryophyllus*) to *Fusarium oxysporum* f. sp. *dianthi*. Phytochemistry 56, 717–721.

Espinosa-García, F.J., Langenheim, J.H., 1991a. Effect of some essential oils phenotypes in coastal redwood on the growth of several fungi with endophytic stages. Biochem. Syst. Ecol. 19, 629–642.

Espinosa-García, F.J., Langenheim, J.H., 1991b. Effect of sabinene and terpinene from coastal redwood leaves acting singly or in mixtures on the growth of some of their fungus endophytes. Biochem. Syst. Ecol. 19, 643–650.

Espinosa-García, F.J., 2001. La diversidad de los metabolitos secundarios y la teoría de la defensa vegetal. In: Anaya, A.L., Espinosa-García, F.J., Cruz-Ortega, R. (Eds.), Relaciones químicas entre organismos: Aspectos básicos y perspectivas de su aplicación. Plaza y Valdéz México, pp. 231–249.

Hernández, A.L., 1997. Evaluación de polvos y aceites vegetales para el control de la mancha púrpura de cebolla *Alternaria porri* en el estado de Morelos. Thesis Universidad Autónoma del estado de Morelos. Mexico, p. 71.

Lira-Saldívar, R.H., Gamboa-Alvarado, R., Villarreal-Cárdenas, L.A., 2001. Plasticidad genotípica de extractos metanólicos de *Larrea tridentata* y su efecto inhibitorio sobre *Fusarium oxysporum* in vitro. Proceedings of the XXVIII National Congress of Phytopathology. Querétaro, México, (Abstr. F-58).

Llanos, A.L., Valdés, E.M.E., Lagunas, O.A., 1993. Control del gusano cogollero del maiz (*Spodoptera frugiperda*), mediante fitoinsecticidas. In: Proceedings of the I International Symposium and II National Meeting on Sustainable Agriculture. Importance and Contribution of Traditional Agriculture. Puebla, Mexico, pp. 244–246.

Merck, 1996. The Merck Index. An Encyclopedia of Chemicals, Drugs and Biologicals. Merck & Co., Inc. USA, p. 900.

Montes, B.R., Cruz, C.V., Peralta, D.M., 1990. Extractos vegetales para el control de la roya del frijol *Uromyces appendiculatus*. Agrociencia protección vegetal. 1, 99–106.

Nigam, S.K., Mitra, C.R., 1970. *Pithecellobium dulce*. V. Chemistry of the seed saponin and constituents of the leaves. Planta Med. 18, 44–50.

Nigam, S.R., Gopal, M., 1997. Pithedulosides A_G oleanane glycosides from *Phitecellobium dulce*. Phytochemistry 44, 1329–1334.

Paulus, A.O., 1990. Fungal diseases of strawberry. HortScience 25, 885–889.

Reyes-Chilpa, R., Gómez-Garibay, F., Moreno-Torres, G., Jiménez-Estrada, M., Quiróz-Vázquez, R.I., 1998. Flavonols and isoflavonols with antifungal properties from *Platymiscium yucatanum* heartwood. Hozforschung 52, 459–462.

Sahu, P.N., Mahato, B.S., 1994. Anti-inflamatory triterpene saponins of *Phitecellobium dulce*: characterization of echinocystic acid bidesmoside. Phytochemistry 37, 1425–1427.

Snowdon, A.L., 1991. A Colour Atlas of Postharvest Diseases and Disorders of Fruits and Vegetables. Fruits, vol. 1. CRC Press, London, p. 302.

Sommer, N.F., Fortlage, R.J., Edwards, D.C., 1992. Postharvest diseases of selected commodities. In: Adel, K. (Ed.), Postharvest Technology of Horticultural Crops. University of California, pp. 117–160.

Southon, I.W., 1994. Phytochemical Dictionary of the Leguminosae. Chapman & Hall, London, p. 1051.

Wagner, H., Bladt, S., Zgainski, E.M., 1984. Plant Drug Analysis. Springer, Berlin, p. 320.