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Evaluation of the Fungicidal Activity of Leaves Powders and Extracts of Fifteen Mexican Plants Against *Fusarium oxysporum* f.sp. *gadioli* (Massey) Snyder and Hansen

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Abstract: *Fusarium oxysporum* f. sp. *gladioli* represent one of the problems of greater importance in the culture of gladiolus. The resistance of this fungus to the fungicides has stimulated the search of new alternatives control measures. The natural plant extracts used in the study were safer to the environment and effective in the control of the plant pathogen tested. The present paper describes the *in vitro* fungicidal or fungistatic effect of powders (20 mg mL^{-1}) and of aqueou, methanol and hexane extracts (5%) of 15 plant species on the development of *F. oxysporum* f. sp. *gladioli* on artificial growth media and volatile compound identification. Twelve plant species showed antifungal activity. The hexane extract of *Chenopodium ambrosioides* (by its fungicidal activity), the methanol extract with *Spondias purpurea* and *Psidium guajava,* as well as the aqueous extract of *L. esculenta* and *Guazuma ulmifolia* inhibited the mycelia growth with a percentage superior to 50%. Also, the powders of *Byrsonima crassifolia* diminished the percentage germination and sporulation of the pathogen. All the species presented antifungal activity in form of methanol extract. The 80% of the plant powders increased the rate of mycelial growth of the fungus. By chromatography of gases and spectrometry of masses, 90 volatile compounds in the powders and extracts were identified that showed activity on the fungus. The majority of the compounds were fatty acids (18.8%), monoterpenes (4.2%), sesquiterpenes (23.6%) and phenolic compound (6.3%). The high chemical diversity of the analyzed plant species, differentially affected the development of the fungus, either for the individual compounds or for synergism of some of them.

Key words: Gladiolus, fungistatic effect, plant species, hexane extracts

INTRODUCTION

Gladiolus is an easy-to-grow flower, especially valued for use in floral arrangements. A number of shades and colors have been developed in Gladiolus flower out of which white, pink and yellow are commonly grown on commercial scale. In Mexico Gladiolus flowers have, become one of the most important of Morelos growers cut flower industry. Fusarium corm rot, or yellows, is caused by the soilborne fungus *Fusarium oxysporum* f. sp. *gladioli.* This disease, generates serious economic losses and increases production costs. In México, this fungus is controlled by intensive fungicide applications that could have result in the development of resistant populations of the pathogens to fungicides, detriment of the human health and contamination of the environment. Therefore, use of plant natural products could be an attractive alternative for the management of this disease. In México, the use of plants extracts with fungicidal potential activity on postharvest fungal disease is scarce (Bautista-Banos *et al*., 2000a, b; Banos *et al*., 2003). Most of plant species have not been investigated for their properties as fungicides for its use in the control of postharvest diseases. A chart of these Mexican plants has been prepared based on the fungicidal or bactericidal background previously reported in the literature. *In vitro* studies carried out by Shaukat *et al.* (2002) reported antifungal activity of *Argemone mexicana* L. against *F. solani* and *Rhizoctonia solani.* Caceres *et al.* (1993) reported that *Byrsonima crassifolia* L. inhibited the growth of *Aspergillus flavus*, *Epidermophyton floccosum, Microsporum gypseum* and *Trichophyton rubrum.* The essential oils of *Ocimum basilicum* L. inhibited *Colletotrichum musae* and *F. proliferatum* (Anthony *et al*., 2004). The aqueous extract of *Persea americana* Miller and *Psidium guajava* L.

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inhibited germination of *Rhizopus stolonifer* (Bautista-Banos *et al*., 2000a) and the development of *C. gloeosporioides* (Banos *et al*., 2002). The specific objectives of the present work were to determine the *in vitro* antifungal activity of 15, Mexican plant species on mycelial growth, conidial germination and sporulation of *F. oxysporum* f.sp. *gladioli* and to identify the volatile compounds.

MATERIALS AND METHODS

Plant material: This study was carried out in the Biotic Products Development Center in Yautepec, state of Morelos, México in January-December of 2008. Fifteen different plant species corresponding to various plant families and including some species reported with medicinal properties were collected in four different natural habitats within the state of Morelos (Table 1). In this four sampling sites the climate is tempered, wet warm and wet tropical with annual precipitancy of 754.6 to 1187 mm. Once harvested, leaves were cut, discarding damaged or diseased material. Plant material was dipped in 1% sodium hypochlorite, rinsed with distilled water, air-dried, macerated with the aid of a blender and a grinder and stored in amber bottles until further use.

Test microorganism: *Fusarium oxysporum* f.sp. *gladioli* were isolated from gladiolus corm rots at Cuautla, Morelos, México and the isolates were maintained on Potato Dextrose-Agar (PDA) in petri plates at temperature 26°C. To maintain biological properties of the fungus, periodic inoculations and reisolations from infected corms were carried out.

Preparation of powders and extracts: To evaluate botanic powders 20 mg mL^{-1} (w/v) of the macerated material was added to plates containing PDA. Leaves powders (50 g) were extracted with hexane, methanol and water (500 mL) for 24 h in each solvent system at room temperature according to Reyes-Chilpa *et al*. (1998). After each extraction step, the leaves extracts were filtered and concentrated in a rotary evaporator (Buchi R-114, Labortechnik Flawil, Switzerland) and then stored at 4°C in amber bottles until use. Plant powder and aqueous extracts were added to PDA, autoclaved (15 lb cm^{-2}, 15 min) and poured into Petri plates (100×15 mm) (Bautista-Banos *et al*., 2000b). The hexane and methanol extracts were added to PDA after sterilization of the media and poured into Petri plates (60×15 mm). A 5 mm agar disc of a 9 days old colony of the pathogen was placed at the center of each plate and incubated at 25°C for 8 to 14 days in the dark. The rate of growth of the fungal colony was calculated as follows: $y = bx + a$, in which b is the slope of the regression line, is the intercept and x is the time of incubation. The percentage of inhibition was $\%I = [(C-T)/C] \times 100$ where, C represents the growth in the non amended control and T in the treatment. There were six replications for each treatment. Control Petri plates contained only PDA and solvent with PDA. Control of PDA was used for comparisons with treated plates. The colony diameter was recorded for each treatment until fungal colonies in the control treatment reached the edge of the plate.

For *in vitro* studies sporulation and germination of spores were measured as previously described by Bautista-Banos *et al*. (2000b). Petri dishes from each treatment were rinsed with 10 mL sterile distilled water, the surface scrapped with a glass rod and the filtrate passed through cotton wool. To test spore viability, a 0.5 mL aliquot of spore suspension was placed on a 20 mm diameter agar disk and after 8 h at room temperature (26°C) stained with lactophenol acid fuchsine. Both, sporulation and germination were determined at 400× magnification. The experiment was repeated twice.

Table 1: Botanical and common namet radiional usase and natural habitat in Morelos, Mexico

Family	Plant specie	Common name	Traditional usage	Natural habitat
Annonaceae	*Annona diversifolia* Saff.	Ilama	Ba	Tetecala
Papaveraceae	*Argemone mexicana* (Sweet) Lindley	Prickly popy	Ba	Yautepec
Malpighiaceae	*Byrsonima crassifolia* (L.) Kunth.	Nance	Ba	Tetecala
Fabaceae	*Caesalpinia pulcherrima* (L.) Swartz.	Bird paradis	Ba	Yautepec
Chenopodiaceae	*Chenopodium ambrosioides* (L.) Webber	Wormseed	Pa	Cuernavaca
Fabaceae	*Erityna americana* Miller.	Erythrina	Ba	Yautepec
Sterculiaceae	*Guazuma ulmifolia* Lam.	Bay cedar	Ba and Pa	Yautepec
Convulvolacea	*Ipomoea murucoides* (Roem.) Schultz.	Cazahuate	Ba	Tetecala
Fabaceae	*Leucaena esculenta* (DC.) Benth.	Guaje	Pa	Tetecala
Lamiaceae	*Ocimum basilicum* L.	Basil	Pa	Amacuzac
Lauraceae	*Persea americana* Miller.	Avocado	Pa	Tetecala
Fabaceae	*Pithecellobium dulce* (Roxb.) Benth.	Huamúchil	Ba and Pa	Yautepec
Myrtaceae	*Psidium guajava* L.	Guava	Pa	Yautepec
Anacardiaceae	*Spondias purpurea* L.	Purple plum	Ba	Tetecala
Meliaceae	*Swietenia humilis* Zucc.	Buzzard	Ba	Tetecala

Traditional usage: Bactericidal (Ba), parasicidal (Pa)

Gas chromatography and spectrometry of masses (CG-MS): In Center of Chemical Investigations of the Autonomous University of the State of Morelos (C.I.Q. U.A.E.M) GC-MS analyses were performed using an Agilent 6890 series GC systems (Agilent Technologies, Santa Clara, CA, USA) coupled to a quadrupole mass spectrometer (model 5973) equipped with an HP5-MS with stationary phase of (5 % phenyl methyl siloxane) capillary column (25 m x 0.20 mm x 0.33 um film thickness). For GC-MS detection electron ionisation with ionisation energy of 70 Ev was used a scan range of 30-550 atomic mass units. Helium was the carrier gas, at a flow rate of 1 mL min^{-1} with a program of temperature of 40°C during 2 min with an increase of 10°C min^{-1} until reaching a temp of 260°C, staying thus during 20 min, work with a way splitters 10:1 and the line of transference of masses MSD to 280°C. For the case of the extracts, a solution of 5 mg in 0.5 mL of reliable the respective one was prepared. In the case of plant powders, before injecting it in the chromatograph, they were entered to the Headspace with a temperature of 100°C, after to the Loop to 120°C and the line of transference registred a temperature of 130°C. The components were identified by matching their recorded mass spectra with the data bank mass spectra (NIST-MS Version 1.7a) and by comparing their relative retention indices with those available in the literature.

Statistical analysis: Powders and plant extracts treatments were arranged in a completely randomized design with six replication. Standard analysis of variance (ANOVA) were used to determine the effects of plant extracts on mycelial growth and spore germination. Treatment means were compared using the Least Sgnificant Difference LSD multiple range test (p = 0.05). Simple linear regression was carried out with data of daily mycelial growth. Sporulation data was analysed using a Kruskal-Wallis (this date did not present normal distribution) test previously to the ANOVA analysis. All the analyses were made in Stat Sigma version 3.5 (Systat Softwere Erkrath, Germany).

RESULTS

There were significant differences in mycelial growth among treatments (p = 0.001) (Table 2), the 80% of plant powders increased the growth rate of the fungus (7.9-8.3). The powders of *B. crassifolia*, *P. guajava*, *S. purpurea* presented the lowest growth rate (5.6-6.9). The aqueous extract of *C. ambrosioides* showed significantly greater growth rate compared to the control. The aqueous extracts of *A. mexicana*, *B. crassifolia*, *G. ulmifolia*, *L. esculenta* and *O. basilicum* showed the lower growth rates (2.4-4.6) with small differences among them. Methanol extracts of all the plant species presented a reduced growth rate compared to the control and ranged 1.7-6.6. Overall, 32 treatments of different powders and extracts diminished significantly ($p \leq 0.001$) the mycelial growth, with a percentage of mycelial inhibition that ranged 8 to 100% and 12 of them showed a mycelial inhibition to at least 40%, particularly methanol extracts, followed of the aqueous and hexane extracts. Among them, the hexane extract of *C. ambrosioides* were the only plant extract that completely inhibited mycelia growth of *F. oxysporum* f. sp. *gladioli*. Also the methanol extracts of *S. purpurea*, *P. guajava* and aqueous extract *L. esculenta* had a percentage of inhibition of 57.2-67 %, respectively (Table 3). Similarly to mycelia growth, there were important differences among treatments on the

Table 2: Effect of powders and extracts of mexican plant on mycelial growth (mm) of *Fusarium oxysporum* f.sp. *gladioli*

Plant species	Powders	Aqueous extract	Methanol extract	Hexane extract
Control	8.5±2.2(7.5)a	85±0 (6.5) a	50±0(6.8)a	49±0.9(6.5) a
Solvent			47.8±1.8(6.6)ab^{ns}	49±0.5(6.2)
Annona diversifolia	8.5±0 (8.3)	71.3±21.6 (5.4) abc^{ns}	46 ±1.5(6.5)bc**	50±0(6.7)
Argemone mexicana	8.5±0 (8.1)	50.4±24.2 (3.4) de***	37±1.1(4.8)d***	49±1.3(6.4)
Byrsonima crassifolia	62.7±2.6 (5.6)c***	62.5±6.8 (4.6) bcd**	26.2±2.9(3.1)g***	50±1.1(6.5)
Caesalpinia pulcherrima	85±0 (8.1)	85.0±0 (6.4)	35.2±2.3(4.5)d***	50±0(6.7)
Chenopodium ambrosioides	85±3.3 (8.1)	85.0±0 (6.8)	35.3±2.1(4.7)d***	0e***
Erythrina americana	85±0 (8.1)	73.8±15.9 (5.5) ab^{ns}	44.2±0a.9(5.7)c	50±(6.7)
Guazuma ulmifolia	85±0 (8.0)	43.4±12.1 (3.0) e***	37±1.1(4.8)d***	49±(6.4)
Ipomoea murucoides	85±0 (8.0)	82.1±7.1 (6.3)	44.7±2.9(6.0)c***	49±1.3(6.4)
Leucaena esculenta	85±0 (8.3)	36.4±14.2 (2.4) e***	31.2±1.1(4.0)e***	49±1.1(6.2)
Ocimum basilicum	85±4.7 (7.9)	59.0±15.4 (4.2) cd***	28.7±3.2(3.5)ef***	29.1±4.4(3.6)cd***
Persea americana	85±0 (8.1)	74.7±8.4 (5.4) ab^{ns}	28.3±3.2(3.5)fg***	29.8±5.6(3.4)d***
Pithecellobium dulce	85±0 (8.1)	80.8±6.6 (6.2)a^{ns}	27.8±1.3(3.4)fg***	50±0(6.9)
Psidium guajava	73.6±1.5 (6.6)b***	75.4±2.7 (5.8) ab^{ns}	21.4±4.3(2.5)h***	41.7±1.4(5.5)b***
Spondias purpurea	77.4±5.8 (6.9)b**	80.5±6.9 (6.3) a^{ns}	16.5±1.4(1.7)i***	30±0.9(3.8)c***
Swietenia humilis	85±0 (8.2)	83.8±3.1 (6.3) a^{ns}	27.4±0.5(3.4)fg***	50±0.8(6.6)
ANOVA	F:23.66 gl:3,20	F:9.5012gl:,64	F:118.88 gl:16,84	F:266.42 gl:5,28

Means followed by different letter(s) in each column are significantly different by LSD test at p = 0.05; Values in parenthesis indicate growth rate in mm day^{-1}*** $p \leq 0.001$,** $p \leq 0.05$; ^{ns}Not significantl; Means without letter were not compared with the control and the rest of the treatments because was same that control

Table 3: Effect of powders and extracts of mexican plant on mycelial inhibition (%) of *Fusarium oxysporum* f.sp. *gladioli*

Plant species	Powders	Wateryextracts	Methanolextracts	Hexane extracts
Control	--	--		--
Disolvente			4.3(1.8)	2(0.5)
A. diversifolia	--	16.1(2.1)	8(1.5)	--
A. mexicana	--	40.7(2.4)	26(1.1)	2(0.5)
B. crassifolia	26.1(2.6)	26.5(0.6)	47.7(2.9)	--
C. ambrosioides	--	--	29.3(2.1)	100(0.0)
C. pulcherrima	--	--	29.72.3)	--
E. americana	--	13.2(1.5)	11.7(0.9)	--
G. ulmifolia	--	48.9(1.2)	26(1.1)	2(0.5)
I. murucoides	--	3.42(0.7)	10.7(2.9)	2(0.5)
L. esculenta	--	57.2(1.4)	37.6(1.1)	2(0.5)
O. basilicum	--	30.6(1.5)	42.7(3.2)	39.2(4.4)
P. americana	--	12.1(0.8)	43.3(3.2)	40.6(5.6)
P. dulce	--	4.9(0.6)	44.3(1.3)	--
P. guajava	13.2(1.5)	11.3(0.2)	57.2(4.3)	15(1.4)
S. humilis	--	1.5(0.3)	45.2(0.5)	--
S. purpurea	8.7(1.8)	5.3(0.6)	67(1.4)	38.8(0.9)

Values in parenthesis indicate SD of the mean

Table 4: Effect of powders and extracts of mexican plant on sporulation (spores mL^{-1}) of *Fusarium oxysporum* f.sp. *gladioli*

Plant species	Powders	Aqueous extract	Methanol extract	Hexane extract
Control	2.13×10^{7} abc	1.25×10^{7} bc	6.84×10^{6} abcde	5.99×10^{6} abcd
Solvent			8.75×10^{6}abcde	8.31×10^{6}abc
Annona diversifolia	2.68×10^{7}abc	7.78×10^{7}ab	1.21×10^{7}abc	1.17×10^{7}abc
Argemone mexicana	7.79×10^{7} a	4.06×10^{7} abc	5.61×10^{6} abcde	9.72×10^{6} abc
Byrsonima crassifolia	6.83×10^{5}	c4.95×10^{7} c	3.45×10^{6} cde	4.97×10^{6} abcd
Caesalpinia pulcherrima	7.18×10^{7}a	4.33×10^{7}abc	9.28×10^{6}abcde	1.19×10^{7}ab
Chenopodium ambrosioides	5.78×10^{7} a b	9.47×10^{7} a	1.82×10^{7} ab	0.0
Erythrina americana	7.12×10^{7} a	2.36×10^{7} abc	1.93×10^{7} a	7.41×10^{6} abcd
Guazuma ulmifolia	6.62×10^{7} a	2.77×10^{7} abc	4.67×10^{6} abcde	3.74×10^{6} bcd
I pomoea murucoides	6.28×10^{7}a	1.05×10^{8}a	1.28×10^{7}abcd	5.81×10^{6}abcd
Leucaena esculenta	2.39×10^{7} abc	1.38×10^{7} bc	5.08×10^{6} abcde	6.68×10^{6} abcd
Ocimum basilicum	5.72×10^{7} a b	5.04×10^{7} ab	3.94×10^{6} bcde	6.00×10^{6} abcd
Persea americana	5.06×10^{7}a b	6.43×10^{7}ab	1.44×10^{7}abc	4.5×10^{6}abcd
Pithecellobium dulce	2.07×10^{7} abc	3.89×10^{7} abc	1.26×10^{7} abcde	1.32×10^{7} ab
Psidium guajava	3.47×10^{7} bc	5.29×10^{6} c	1.13×10^{6} de	1.17×10^{6} cd
Spondias purpurea	2.32×10^{7} abc	4.16×10^{7} abc	3.23×10^{5} e	1.61×10^{6} d
Swietenia humilis	3.15×10^{7}abc	3.0×10^{7}abc	6.67×10^{6}abcde	1.44×10^{7}a
ANOVA	H:67.35 gl:15	H:75.90 gl:15	H:72.69 gl:16	H:71.28 gl:15

Means followed by different letter(s) in each column are significantly different by Dunn`s test at p = 0.05

sporulation of the pathogen (p = 0.001) (Table 4), the treatments that produced the lower number of spores mL^{-1} as compared to the control were powders of *B. crassifolia* and aqueous extracts of *P. guajava*. Aqueous extract of *A. diversifolia, C. ambrosioides*, *I. murucoides*, and *P.americana*, produced a significantly higher (p = 0.05) number of spores mL^{-1} as compared to the control. The sporulation of *F. oxysporum* f. sp. *gladioli* was reduced significantly (p = 0.05) by eight methanol extracts (*A. mexicana*, *B. crassifolia*, *G. ulmifolia*, L. *esculenta*, *O. basilicum, P. guajava, S. purpurea* and *S. humilis*) and ranged 3.23×10^{5} to 5.61×10^{6} spores mL^{-1}. Methanol extracts in where the higher number of spores mL^{-1} was obtained were those of *A. diversifolia*, *C. pulcherrima, C. ambrosioides, E. americana, I. murucoides, P. americana* and *P. dulce* (Table 4).

The hexanic extracts of *B. crassifolia, C. ambrosioides, G. ulmifolia, I. murucoides, P. americana, P. guajava* and *S. purpurea,* had a lower number of spores mL^{-1} with respect to control treatments (0.0 to 5.81×10^{6} spores mL^{-1}). On the opposite, hexanic extract of *A. mexicana, A . diversifolia, C. pulcherrima, E. americana, P. dulce* and *S. humilis* had a higher number of spores mL^{-1} that the control treatment (5.99×10^{6} spores mL^{-1}). Hexanic extract of *C. ambrosioides* inhibited the sporulation completely.

Percentage of *F. oxysporum* f. sp. *gladioli* conidial germination differed significantly among experimental treatments ($p\leq0.001$). Powders of *B. crassifolia* (27.8%) and *S. humilis* (32.2%) had the lower percentage of spore germination. Powders of *I. murucoides, A. mexicana, P. dulce, P. americana* and *C. pulcherrima* was significantly lower than that on the control and ranged from 6.33 to 72.9%.

The percentage of conidial germination of *F. oxysporum* f. sp. *gladioli* was 52.0, 59.6, 60.1 and 60.6% with aqueous extracts of *S. humilis, C. pulcherrima, P. dulce* and *S. purpurea,* respectively. The treatment of

S. humilis presented the lower average of germinated conidia (p = 0.05) in the experiment. In addition, treatments with lower percentage of germination that on the control (p = 0.05) were the methanol extract of *G. ulmifolia, S. humilis, B. crassifolia, A. mexicana, S. purpurea* and *C. pulcherima,* with percentage ranging 62.4 to 86%. Seven of those treatments of the methanol extracts, had a higher percentage of germination than the control. Concerning hexanic extracts, six treatments reduced (p = 0.05) germination level on the control, these treatments included extracts of *C. pulcherrima, I. murucoides, S. purpurea, L. esculenta, A. diversifolia* and *P. dulce*, with percentage of germination from a 57.8 to 75.7%. Treatments with *O. basilicum* (96.9%) and *B. crassifolia* (94.8%) reached the highest number of germinated conidia. On the opposite, treatment *G. ulmifolia* reached only 34.4 % of germination, and nil conidia germinated with *C. ambrosioides* (Table 5).

In agreement with the effect obtained on the mycelial growth of *F. oxysporum* f. sp. *gladioli*, 12 methanol extracts, four hexane extracts, five aqueous extracts and the powders of three plant species were selected to chromatographic analysis. In powders and extracts 90 compounds were identified, which, belong to

Table 5: Effect of powders and extracts of mexican plants on conidial germination (%) of *F. oxysporum* f.sp. *gladioli* after 8 h incubation at 25°C

	Conidial germination (%)			
Plant species	Powders	Aqueous extract	Methanol extracts	Hexane extracts
Control	90.7±34 ab	93.9±7 a	90.2±12 bcd	86.8 ±10 cd
Solvent			87.6±16 cd ns	89.1 ±39bcd ns
Annona diversifolia	99.6±2 a ns	80.0±31 cd ***	97.1±3 a *	73.3 ±17 e ***
Argemone mexicana	63.3±20 d***	89.3±11 a^{ns}	68.5±28 g***	85.9 ±15 cd^{ns}
Byrsonima crassifolia	27.8±17 e ***	76.6±13 de ***	65.9±20 g ***	94.8 ±8 ab *
Caesalpinia pulcherrima	72.9±28 cd*	59.6±22 f***	78.6±16 ef***	57.8 ±16 g***
Chenopodium ambrosioides	90.4±44 ab^{ns}	68.9±34 e ***	94.7±12 ab^{ns}	0.0±0h ***
Erythrina americana	82.3±34 bc^{ns}	94.6±11	85.1±30 de^{ns}	90.4 ±3 abc^{ns}
Guazuma ulmifolia	86.6±22 ab^{ns}	73.8±22 de ***	62.4±21g ***	34.4 ±114
Ipomoea murucoides	67.8±92 d ***	96.7±6	97.2±6 a*	65.2 ±15 f ***
Leucaena esculenta	86.8±22 ab^{ns}	77.2±16 cd ***	97.2±3 a *	70.8 ±13 ef***
Ocimum basilicum	87.4±27 ab^{ns}	91.7±11 a^{ns}	92.2±16 abc^{ns}	96.9 ±4 a *
Persea americana	72.6±23 cd *	84.1±6 ab *	96.9±7 a *	91.2 ±18 abc^{ns}
Pithecellobium dulce	72.3±36 cd*	60.1±11 f***	95.4±10 ab^{ns}	75.7 ±25 e***
Psidium guajava	90.7±12 ab^{ns}	84.4±38 ab *	84.6±21 de^{ns}	85.6 ±15 cd^{ns}
Spondias purpurea	89.3±32 ab^{ns}	60.6±10 f***	77.3±27 f***	70.4 ±14 ef***
Swietenia humilis	32.2±16 e ***	52.0±25 g ***	64.5±15 g ***	82.8 ±23d^{ns}
	F:18.91 gl:15,80	F:21.342gl:13,69	F: 27.374 gl:16,82	F: 96.139gl:15,78

Means followed by different letter(s) in each column are significantly different by LSD test at p = 0.05; *** p≤0.001;* p≤0.05 ^{ns}Not significantly

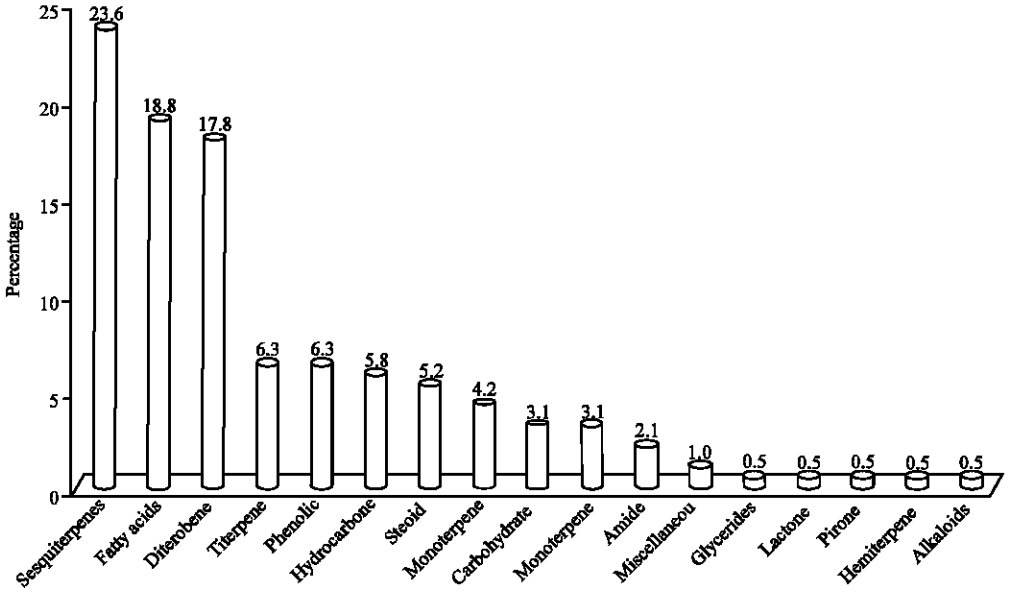

Fig. 1: Volatile compounds in the powders and extracts identified by chromatography of gases and spectrometry of masses

17 families of compounds which include, sesquiterpenes (23.6%), fatty acids (18.8%), hydrocarbons (5.8%), phenolic compounds (6.3%), monoterpenes (4.2%) and diterpenes (17.8%). Most of identified compounds were soluble in methanol and hexane (Fig. 1); the detected volatil compounds had molecular weights of between 126 to 500 MW. The diterpenes, triterpenes and fatty acids were present in most of the species. On the contrary, the sesquiterpenes were detected only in *O. basilicum*, *P. american, P. guajaba, S. humilis* and *S. purpurea*. The sesquiterpenes were not detected in *A. mexicana, B. crassifolia, C. ambrosioides, C. pulcherrima, G. ulmifolia* and *L. esculenta*.

DISCUSSION

The effect of different powders and evaluated extracts, was different according to the stage of development of *F. oxysporum* f. sp. *gladioli*, being the most sensitive the mycelial growth. This detrimental effect on mycelial growth must be caused by fatty acids and terpenes presents in the extracts, break of the cellular membrane caused by lipophilic compounds (Cowan, 1999), as most of the species that showed activity against the fungus contained these type of compounds. The methanol extracts of all the plant species tested showed a higher percentage of mycelial inhibition compared with aqueous extracts. This agrees with the report of Alanis *et al*. (2005) that consider the methanol extracts presented greater activity than the aqueous. This activity must be related to the type of compounds and not to the dissolvent used in the extraction process (Askun *et al*., 2009) but also the strong capacity of extraction of methanol compared to water (Bhattacharjee *et al*., 2006). With respect to the effect of the extracts on the conidial germination of *F. oxysporum* f. sp. *gladioli*, was the opposite to mycelia growth, the aqueous extracts that have a greater detrimental effect in comparison to the methanol extracts, indicating that the effect of some plant species varies with the fungal structure or stage of development of the pathogen. Montes- Belmont and Prados-Ligero (2006) observed that some extracts affected the mycelial growth of *S. cepivorum*, whereas others plant species, affected the formation of sclerotia. In the case of the hexane extracts, Qin *et al*. (2009) one nonpolar dissolvent extract lipophilic substances, obtaining mainly fatty acids. In our study, the hexane extract of *C. ambrosioides* had a fungicidal effect (complete inhibition of fungal growth) on *F. oxysporum* f. sp. *gladioli*. Other works reported a reduction of the rate of mycelial growth when applying the essential oils of this plant on *F. oxysporum* f. sp. *gladioli* (Barrera-Necha *et al.,* 2009) and *F. oxysporum* (Kumar *et al*., 2007). Reported that the aqueous extracts of this species had a fungistatic effect on *A. flavus* and an inhibiting effect on its germination. In the present work, the aqueous extracts inhibited the germination of *F. oxysporum* f. sp. *gladioli*. Another important compound is the Ascaridole presented in the methanol and hexanic extract of *C. ambrosioides* in a percentage of 8,92% unlike the methanol extract with only 2,52%. The presence of this compound in the nonpolar extract and the extract of average polarity as is methanol, agrees with MacDonald *et al*. (2004), who mention that this compounds are extracted mainly with hexane. Rimada *et al*. (2007) reports that ascaridole is the major active principle of this specie. The hexane extracts of *C. ambrosioides* had greater number of monoterpenes compound (Pulegone, Ascaridole and Geraniol tiglato) in comparison to the methanol (Table 6), which could increase the activity of ascaridole. The β-sitosterol (10.06%) and δ-sitosterol (24.49 %) were identified only in methanol extracts, previous research reported antifungal activity of these compounds (Kiprono *et al*., 2000; Ghazala *et al*., 2004). The relation between composition and antifungal activity of these plant extract may be attributable both to their main more abundant components but also to that presents in small amount. It is possible that they may act synergistically to contribute to the toxicity of the totality of the tested plant extract. Powders, methanol and aqueous extracts of *B. crassifolia* had effect on the growth, sporulation and germination of the fungus. The major compounds in the methanol extracts was linoleic acid, a fatty acid that has antimicrobial properties (Santoyo *et al*., 2006). Powders, methanol and hexanic extract of *P. guajava* inhibited the mycelial growth and sporulation. The methanol extracts included mainly terpenes and fatty acids, groups considered with antimicrobial activity (Cowan, 1999). Methanol extract and powders of *S. purpurea* inhibited mycelial growth of *F. oxysporum* f. sp. *gladioli* and hexanic extract the sporulation. These extracts are characterized by the presence of several terpenes, fatty acids and phenolic compounds which has antimicrobial activity (Deans *et al*., 1995). With extracts obtained from leafs of this specie Bautista-Banos *et al*. (2000a) reported inhibition in the germination of *R. stolonifer*. Methanol extract of *P. americana* inhibited the mycelial growth of *Fusarium* sp. in 43.3%, this plant specie, completely inhibited to *C. gloesosporoides* (Banos *et al*., 2002), and also showed antibacterial activity (Pasewu *et al*., 2008; Castillo-Juarez *et al*., 2009). The presence of sesquiterpenes in *P. guajava, S. purpurea* and *P. americana* would be responsible for its antifungal

Table 6: Percentage composition of volatile compounds of methanol and hexanic extracts of *Chenopodium ambrosioides*

			Methanol		Hexanic	
Compounds	t(min)	Mw	Área	%	Área	%
Pulegone	10.94	152			94778355	1.21
3- Carene	12.84	136	51940776	5.00	1705741153	21.75
Ascaridole	13.84	168	26136147	2.52	699589872	8.92
9,12,15- Octadecatrienoic acid, 2 -(acetiloxi)-1-(acetiloxi, met hyl) et hyl ester	16.41	430			272012011	3.47
2(4H) -Benzofuranose, 5,6,7,7a-tetrahidro- 4,4,7a-trimetil(dihidroactinolide)	16.96	180			70613682	0.90
Gerani ol tiglate	18.34	236			69180941	0.88
Miristic acid	19.50	228			72767871	0.93
Fit 1, 6 diene	20.08	<300	95757022	9.22	93504263	1.19
Fitone (Perhidrofarnesil acetona)	20.10	268			128363169	1.64
Ciclotetradecane	20.52	196			128776213	1.64
Fit 2, 6 diene	20.54	<300	31390266	3.02		
Hexadecanoic acid, methyl ester	20.98	270	23511428	2.26		
Hexadecanoic acid	21.59	256	38060378	3.66	1713791313	21.85
Isophytol	22.91	296	100445875	9.67	320605890	4.09
Oleic acid	23.33	282			1267532001	16.16
Linoleic acid	23.31	280	312550730	30.09		
Tetracosane	25.44	338			39730941	0.51
Hexatriacontane	26.70	500			57651204	0.74
Stearic, 2-(9-octadeceniloxy) ethy l ester	30.40	570			980983117	12.51
β-sitosterol	32.79	414	104443786	10.06		
γ- Sitosterol	32.85	414	254410756	24.49		
Squalene	33.75	410			127702683	1.63
Total			1038647164	100	7843324679	100

activity. This hypothesis is consistent with Chang *et al.* (2008) who mentioned that antifungal activity of sesquiterpenes constituents were superior to monoterpenes constituents against different fungi. Reported that the oil of *Curcuma longa* which consists mainly of sesquiterpenes exhibited a complete mycelia growth inhibition against *F. oxysporum.*

Aqueous extract of *L. esculenta* inhibited mycelial growth in 57%, but antifungal or antimicrobial activity have not been reports previously. *S. humilis*, *G. ulmifolia* and *A. mexicana* (aqueous) inhibited mycelia growth and germination of *F. oxysporum* f. sp. *gladioli*. Previous studies have demonstrated the antibacterial activity of *G. ulmifolia* (Argueta, 1994; Camporese *et al*., 2003) and antifungal activity of *A. mexicana* (Shaukat *et al*., 2002; Masood and Ranja 1991). Methanol extracts of *P. dulce* inhibited the mycelial growth of *F. oxysporum* f. sp. *gladioli* in 47,7% and the germination in a 40% (aqueous extract). Barrera-Necha and Bautista-Banos (2002) observed that powders of seeds of *P. dulce* inhibited mycelial growth of *F. oxysporum* only 4,9%, and at 10 mg mL^{-1} stimulates the growth of the fungus. Different studies exist on their activity against pathogenic fungi, as *Uromyces appendiculatus* (Montes *et al*., 1990), *Botrytis* sp., *Rhizopus stolonifer* and *Penicillium digitatum*, in form of powders and etanolic extracts (Necha *et al*., 2002; Banos *et al*., 2002, 2003) and against *C. gloeosporioides* (Peraza-Sanchez *et al*., 2005). Results of this research demonstrated the antifungal potential activity of a range of plant species that could be used as potential antifungal agents for the control of fungal diseases in ornamental plants. Future research will include the isolation of the active compounds of those extracts with fungicidal properties.

CONCLUSION

Present data support the possible use of extracts of the tested plant species, in particular *C. ambrosioides*, against one important pathogenic fungus as *F. oxysporum* f. sp. *gladioli*. Such products of plants would be biodegradable, renewable in nature and safe to human health.

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