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## ***Flourensia cernua* DC: A Plant from Mexican Semiarid Regions with a Broad Spectrum of Action for Disease Control**

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### **1. Introduction**

Mexico has an extensive variety of plants, it is the world's fourth richest country in this aspect. Some 25,000 species are registered, and it is thought that there are approximately 30,000 not described. Particularly the regions of the north of Mexico, with their semiarid climate, have a great number and variety of wild plants grown under extreme climatic conditions. Wild species which have compounds with flavonoid structures, sesquiterpenoids, acetylenes, *p*-acetophenones, benzofurans, and benzopyrans grow in these regions. The polyphenolic compounds include tannins and flavonoids which have therapeutic uses due to their anti-inflammatory, antifungal, antibacterial, antioxidant, and healing properties.

*Flourensia cernua* is an endemic species which grows in semiarid zones of Mexico and contains polyphenolic, lactone, benzofuran, and benzopyran compounds which give it a potential use for disease control.

In this work, *F. cernua* is reviewed in terms of its geographical distribution in Mexico, traditional uses, bioactive compounds identified for controlling fungi, bacteria, and insects, as well as cytotoxic activity.

### **2. Common names**

It is commonly known in different ways, as it is found in the United States of America as well as in Mexico. The names given in the United States of America are: tarbush, hojase, American-tarbush, black-brush, varnish-brush, and hojasen (Correl and Johnston, 1970; Vines, 1960). In Mexico, it is known as hojasen, tarbush, black-brush (Arredondo, 1981).

### 3. Geographical distribution

In Mexico, *F. cernua* is found in the Chihuahuan and Sonoran deserts, as well as in the states of Coahuila, Chihuahua, Durango, Hidalgo, Nuevo León, San Luis Potosí, Sonora, and Zacatecas (Valdés, 1988; Martínez, 1993) (Fig. 1). In the United States of America, it is found West of Texas and South of New Mexico and Arizona (Vines, 1960).

It is found in altitudes ranging from 1000 to 2000 masl. In studies carried out in Mexico, it is observed that the prevailing altitude for this species is 1900 masl and slopes from 1 to 6 per cent (Arredondo, 1981) although it is also found in altitudes from 300 to 400 Northeast of Coahuila.

### 4. Associated species

The main shrub species to which *Flourensia cernua* (Fig.2) is related to are: *Larrea tridentata*, *Yucca filifera*, *Atriplex canescens*, *Castela texana*, *Acacia farnesiana*, *Prosopis juliflora*, *Agave lechuguilla*, *Parthenium incanum*, *Fouquieria splendens*, and *Acacia constricta* (Comisión Técnico Consultiva para la Determinación de los Coeficientes de agostadero, 1979).

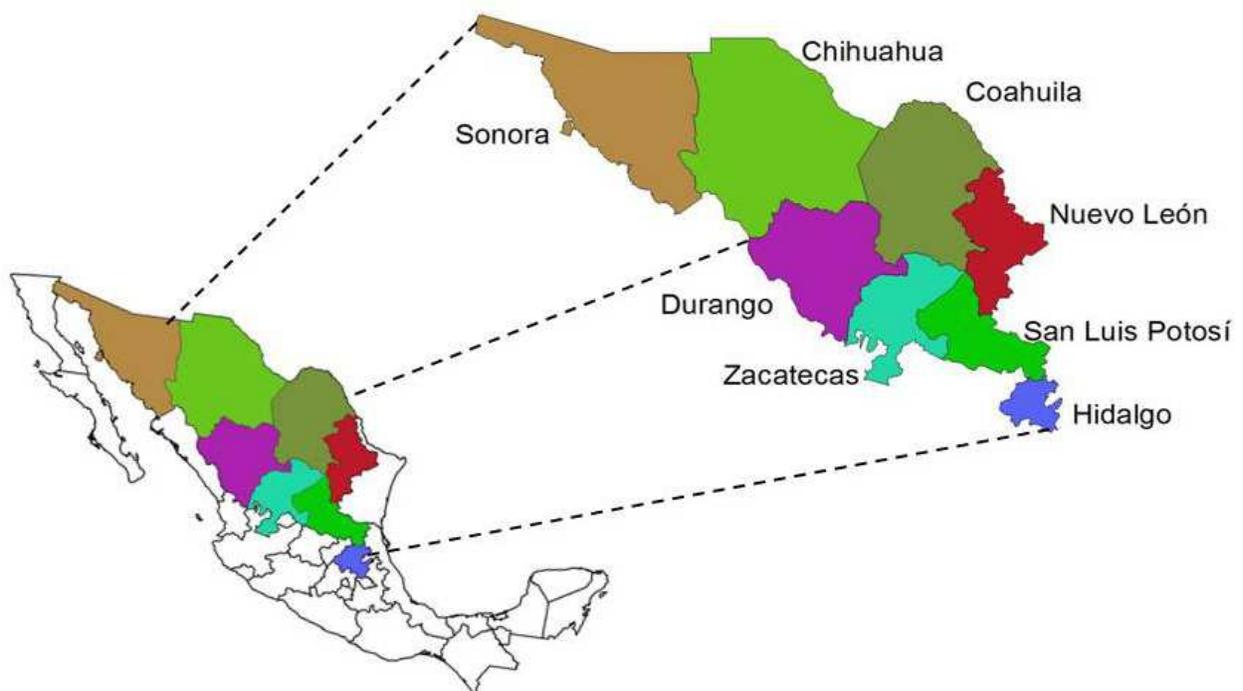


Fig. 1. Geographical distribution of *Flourensia cernua* DC in Mexico.



Fig. 2. *Flourensia cernua* plant at su in a wilderness site in Northeast Mexico.

## 5. Phytochemical analysis

The *Flourensia* genus is important due to the great amount of secondary metabolites it possesses; these are widely used for biological and ecological applications. Nine species of *Flourensia* have been reported, being *Flourensia cernua* the one with the highest number of chemicals (Aregullín and Rodríguez, 1983) with economical potential. The authors correlated the presence of benzofurans and benzopyrans with biological activity. The fact that these secondary metabolites are not present in other species, led to a correlation between the ecographical distribution and a possible chemical adaptation to the environment.

## 6. Properties and documented actions

Several medicinal properties have been reported for the tea obtained from the leaves or flowers for indigestion and gastrointestinal problems (Arredondo, 1981).

The green fruits are innocuous for cattle. However, dry fruits are toxic and when consumed at approximately 1% of the animal weight they cause death during the first 24 hours (Sperry et al., 1968).

## 7. Active chemicals

Fractionation of a  $\text{CH}_2\text{Cl}_2$ -MeOH (1:1) extract of the aerial parts of *Flourensia cernua* led to the isolation of three phytotoxic compounds, namely dehydroflourensic acid (Fig.3a), flourensadiol (Fig.3b), and methyl orsellinate (Fig.3c) and seven hitherto unknown  $\gamma$ -lactones were obtained (Fig.3d), these being tetracosane-4-olide, pentacosane-4-olide, hexacosane-4-olide, heptacosane-4-olide, octacosane-4-olide, nonacosane-4-olide and triacontane-4-olide. Besides, a previously known flavonoid, ermanin (Dominguez et al., 1973). Also there are benzopyrans (Fig.3e) and benzofurans (Fig.3f).

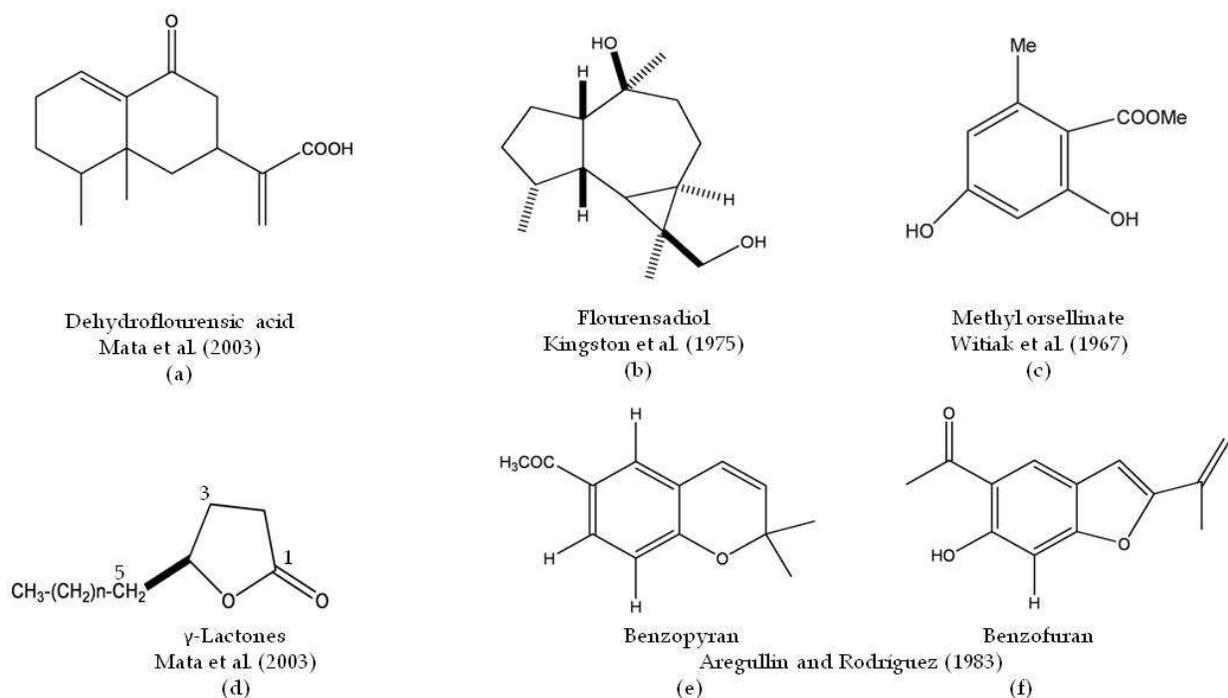


Fig. 3. Chemical of six active compounds from *Flourensia cernua*.

## 8. Plant fungicide, bactericide, and insecticide activity

### 8.1 Antifungal activity

Fungicide activity *in vitro* of leaves extracts at solution concentration of 1,000 mg L<sup>-1</sup> on *Rhizoctonia solani*, *Pythium* sp. and *Fusarium oxysporum* was reported (Saedi-Ghomi & Maldonado, 1982). The leaf fractions of hexane, diethyl ether and ethanol were active against *Colletotrichum fragariae* Brooks, *C. gloesporioides* Penz and Sacc. The essential oils from the hexane fraction were active at 1  $\mu$ g doses, whereas the diethyl ether and ethanol fractions were active at 10  $\mu$ g doses. The ethanol fraction was active against *C. acutatum* Simmons only at 400  $\mu$ g. (Tellez et al., 2001).

Gamboa et al. (2003) used an extraction method by soxhlet to obtain methanolic extracts which were evaluated on soil pathogene *Rhizoctonia solani* and on phytopathogene algae *Phytophthora infestans*, 20,000  $\mu$ l L<sup>-1</sup> were the required dose for 86% pathogene inhibition.

Mata et al. (2003) reported that the fractionation of an extract of the aerial parts of *F. cernua* led to the isolation of three phytotoxic compounds namely: Flourensadiol, methyl orsellinate, and dehydroflourensic acid.

In a study carried out at our lab the inhibitory effect of ethanolic extracts was evaluated for three *Flourensia* species: *F. cernua*, *F. microphylla*, and *F. retinophylla* on three pathogens: *Alternaria* sp., *Rhizoctonia solani* (Fig.4), and *Fusarium oxysporum*, which attack commercial cultivars (Jasso de Rodriguez et al., 2007). The variance analysis on the pathogen mycelial development showed highly significant differences ( $p \leq 0.01$ ) on extract, dose, and extract interactions x dose. *F. microphylla* inhibited *Alternaria* sp 42.5% at a 10  $\mu$ l L<sup>-1</sup>, reaching 76.8% at a 100  $\mu$ l L<sup>-1</sup>. *F. cernua* and *F. retinophylla* showed a similar effect for high concentrations,

however, inhibition was slightly lower than *F. microphylla* at low concentration. The highest inhibition level was 98.6%.

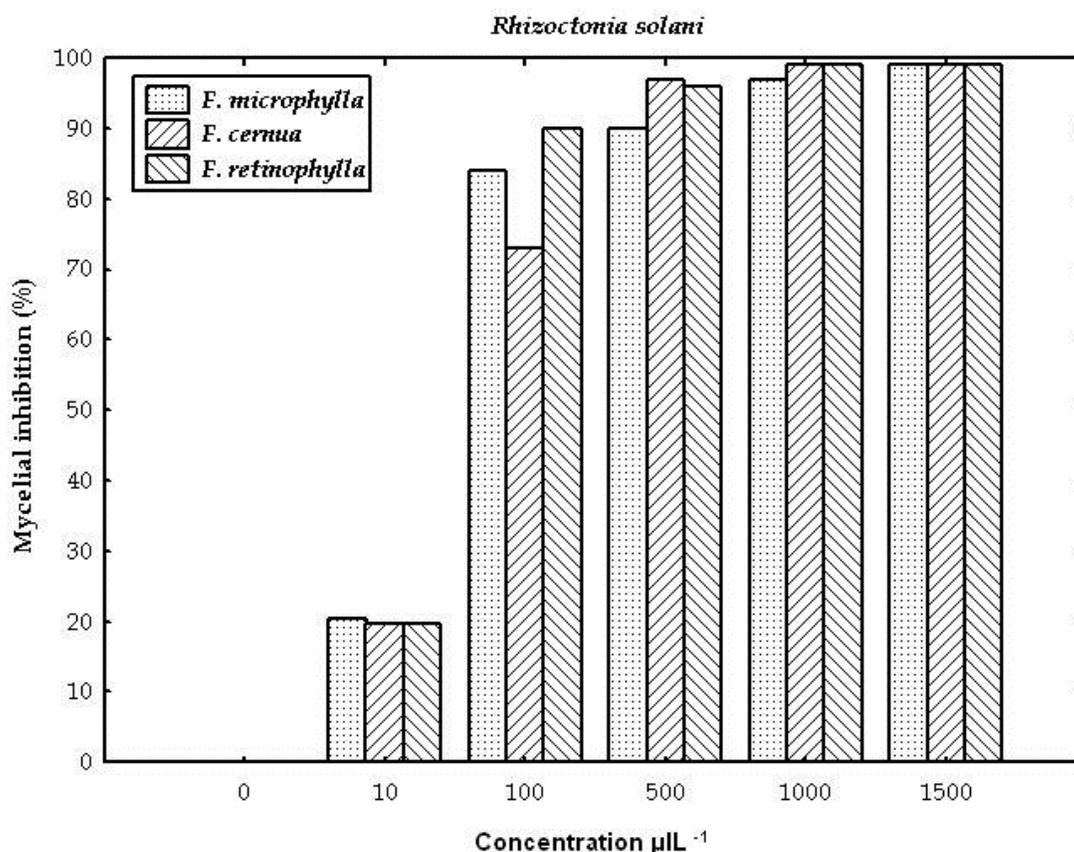


Fig. 4. Mycelial growth inhibition (percentage of *Rhizoctonia solani*) as a function of *Flourensia* spp. extracts concentration.

In studies carried out by our research group, Guerrero-Rodriguez et al. (2007) proved the *F. cernua* effect on mycelial inhibition of *Alternaria alternata*, *Colletotrichum gloesporoides*, and *Penicillium digitatum*, where methanol:chloroform (1:1) solvents and sequential extractions with hexane, diethyl ether, and ethanol were used. *A. alternata* reported the highest mycelial inhibition, took place with hexane fractions (91.9%) and methanol: chloroform (88.4%) at 4000 mg L<sup>-1</sup>. 2000 mg L<sup>-1</sup> ethanol extract caused the lowest production of *C. gloesporoides* conidium. The four extracts reduced conidium production for *P. digitatum*, however they didn't present statistical differences. In general, the ethanolic extract was the most efficient for inhibiting mycelial growth of *C. gloesporoides* and *P. digitatum*, (Fig. 5).

Studies carried out in the UAAAN by Castillo et al. (2010) on plant extract of *Larrea tridentata*, *Flourensia cernua*, *Agave lechuguilla*, *Opuntia* sp, and *Yucca* sp; obtained with alternative organic solvents (lanolin and cocoa butter) and water were tested against *Rhizoctonia solani* pathogens. The obtained results were as follows: The *L. tridentata* and *F. cernua* extracts by means of lanolin and cocoa butter at a 2000 and 1000 ppm total tannins inhibited the growth of *R. solani* 100%. Lanolin and cocoa butter solvents allowed a high recovery of polyphenolic molecules of strong antifungal activity against *R. solani* thus offering an alternative production of antimicrobial agents.

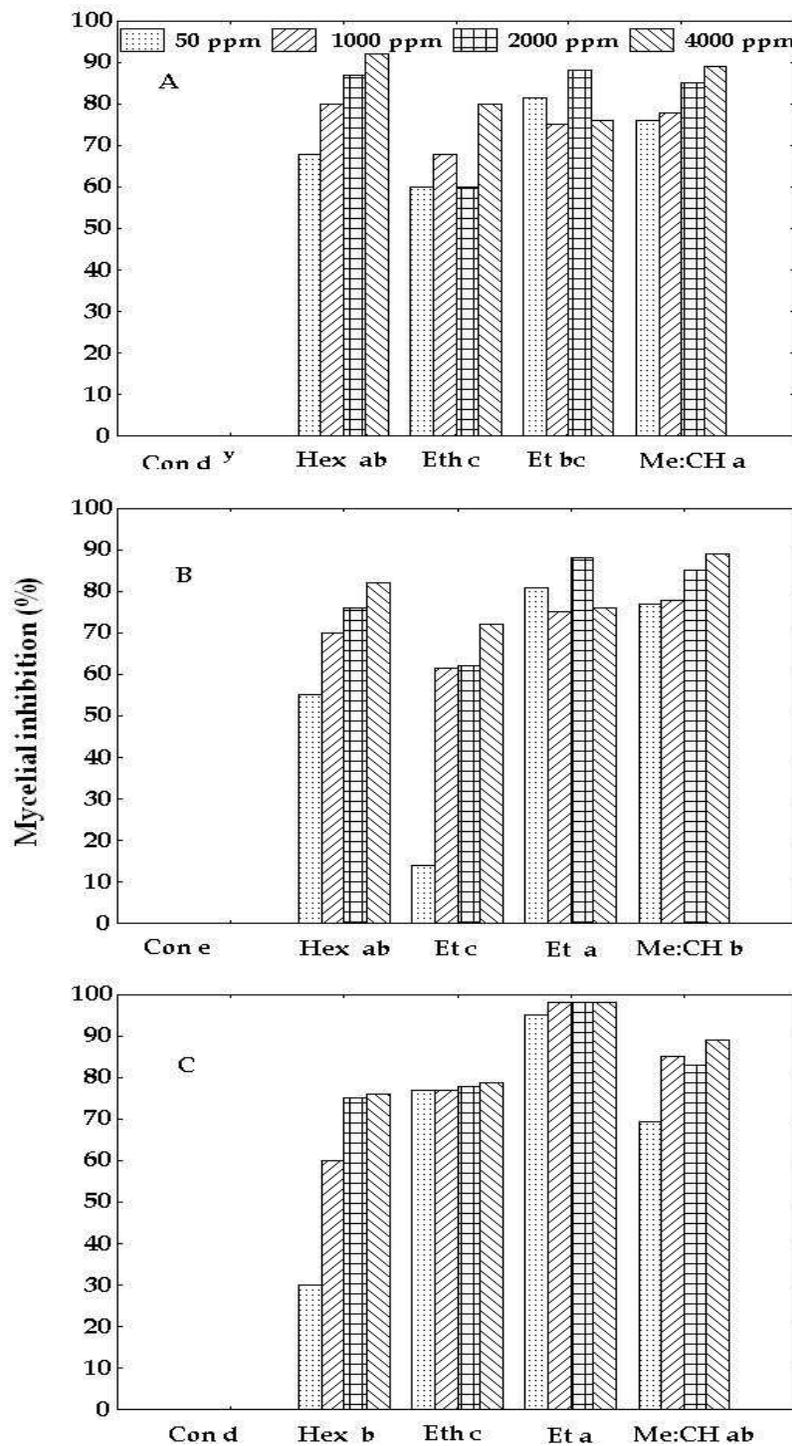


Fig. 5. Percentage of mycelial inhibition of four extracts from *Flourensia cernua* DC at four concentrations on three postharvest pathogens: A) *Alternaria alternata*, B) *Colletotrichum gloeosporioides*, and C) *Penicillium digitatum*.  $\gamma P \leq 0.01$  (LSD). Con=Control; Hex=Hexane; Eth=Ether; Et=EtOH, and Me:CH=MeOH-CHCl<sub>3</sub>.

## 8.2 Antibacterial activity

The mixtures of benzofurans and benzopyrans of *F. cernua*, were tested against Gram positive and Gram negative bacteria, fungi and *Saccharomyces* under two experimental

conditions: One where the inoculated media was kept in darkness and the other one where the inoculated media was UV irradiated (280-400 nm) for 15 min previous to incubation in darkness. Bioactivity was greatly increased by the UV irradiation (Aregullin & Rodriguez, 1983; Towers et al., 1975).

Molina-Salinas et al. (2006), evaluated the crude extracts effect of methanol, acetone, and hexane of the aerial parts of *Artemisa ludoviciana* Nutt., *Chenopodium ambrosioides* L., *Murrubium vulgare* L., *Mentha spicata* L., and *Flourensia cernua* DC to inhibit the growth or death of *Mycobacterium tuberculosis* strains H37Rv and CIBIN:UMF:15:99. Results showed that from the evaluated plants, *F. cernua* was the only active plant which inhibited and killed *Mycobacterium tuberculosis* strains H37Rv and CIBIN:UMF:15:99. The hexane extract showed a minimal inhibitory concentration (MIC) 50 and 25  $\mu\text{g L}^{-1}$  against sensitive and resistant strains, respectively; acetone extract was active against and only CIBIN:UMF:15:99 (MIC=100  $\mu\text{g L}^{-1}$ ). It may be concluded that hexane extract of *F. cernua* leaves could be an important source of bactericidal compounds against multidrug-resistant *M. tuberculosis*.

In a study carried out by our research group, the antibacterial activity of *F. cernua* obtained with hexane, ether, ethanol, and metanol-chloroform mixture, at different dose, was evaluated on *Pseudomonas cichorii* (Pc), *Xanthomonas axonopodis* pv. phaseoli (Xap), and *Pectobacterium caratovora* subsp. atroseptic (Pca) (Peralta, 2006). All the extracts showed activity on Xap and Pc, however, none of them showed any inhibition effect when evaluated on Pca. *F. cernua* hexane extract at a 4000  $\mu\text{l ml}^{-1}$  ( $P \leq 0.05$ ) concentration showed the highest inhibition on Xap (82.51%) and Pc (83.96%). The other extracts showed a lower activity (Table 1).

Extracts and concentrations ( $\mu\text{l L}^{-1}$ )	Inhibition of CFU $\text{ml}^{-1}$								
	Pc	A	B	Xap	A	B	Pca	A	B
Hexane	500	1.89		a	37.23		a	0	a
	1000	26.41	B	ab	51.55	C	ab	0	A a
	2000	44.34		b	74.77		b	0	a
	4000	83.96		c	82.51		c	21.73	B
Diethyl ether	500	0		a	2.41		a	11.01	c
	1000	12.95	A	ab	0	B	a	5.29	A b
	2000	17.27		b	2.08		a	0	a
	4000	47.48		c	63.44		b	3.96	Ab
Ethanol	500	18.7		a	0		a	4.58	a
	1000	20.87	A	ab	0	A	a	5.6	B ab
	2000	43.04		b	1.47		a	13.49	c
	4000	52.17		c	14.73		b	9.92	B
Meth-Chlor	500	0		a	0		a	5.93	c
	1000	6.77	A	b	0	A	a	3.56	A a
	2000	0		a	17.46		b	3.86	a
	4000	0		a	0		a	4.91	b

Table 1. Interactions of *F. cernua* extracts x concentrations of the CFU  $\text{ml}^{-1}$  inhibition percentage, of three bacteria at 24 h of incubation. Meth-Chlor= Methanol-Chloroform; Pc = *Pseudomonas cichorii*; Xcp = *Xanthomonas axenopodis* pv. Phaseoli; Pca = *Pectobacterium caratovora* subsp. Atroseptica; A: Extract, \*\* =  $p \leq 0.05$ ; B: Concentration \*\* =  $p \leq 0.05$ .

### 8.3 Insecticide activity

The insecticide activity of the benzofuran 7-methoxy-2-isopropenyl-5-acetyl-2, 3-dihydrobenzofuran-3-ol-cinnamate proved its activity as a juvenile hormone causing anatomic malformation, juvenile characteristics retention and sterility in the insects treated from their second to fourth stages of development (Towers et al., 1975). The results were similar to those reported by Bowers (1971) with precosene. Termiticidal activity of hexane, diethyl ether and ethanol fractions was found by Tellez et al. (2001).

In lab studies carried out by our research team, when evaluating the bioinsecticide activity of crude extracts from *F. cernua* leaves extracted with solvents of variable polarity on three insect plagues of agronomical importance: *Sitophilus oryzae* (Linnaeus), *Phthorimaea operculella* (Zeller), and *Brevicoryne brassicae* (Linnaeus), as well as the repellent or attraction effect on *Sitophilus oryzae* (Linnaeus) (Martinez, 2006). The following results were obtained: extracts didn't provoke mortality at 24 and 48 h ( $P \leq 0.05$ ) on *Sitophilus oryzae* (Linnaeus), *Phthorimaea operculella* (Zeller); mortality for cabbage plant louse (*B. brassicae*), by effect of all extracts was observed, although some required a higher concentration to kill beings 100%. The extract that presented insecticide effect potential against *B. brassicae* was hexane which had 100% mortality from concentration at 10,000  $\mu\text{l L}^{-1}$  ( $P \leq 0.05$ ) at 24 h. Besides, hexane fraction showed insectistatic effect when inciting repellency to *S. oryzae* at 5 and 45 days, in raffia sacks as well as in jute sacks (Fig. 6). The repellency effect incited by the hexane fraction may be due to the volatile substances borneol and camphor it contains.

### 8.4 Antioxidant activity

Salazar et al. (2008) in a study carried out in order to evaluate the antioxidant potential of six species of the Northeast of Mexico, reports that leaves, stem, root, and flowers of *F. cernua* possess antioxidant activity, due to the content of phenolic compounds showing up in the different parts of the plant. Stems and roots of this species report the highest contents of phenolic compounds.

### 8.5 Cytotoxic activity

Pure benzopyrans and benzofurans of *F. cernua* (Figs. 3 e and f) have been studied for cytotoxic activity using blood red cells and measuring the hemoglobin released on cell destruction. The benzopyrans were more active than benzofurans although no clear correlation between activity and structure has been obtained. The UV irradiated compounds showed higher cytotoxic activity than the non-irradiated ones (Towers et al., 1980).

Benzopyrans and benzofurans react with L-cystein (Towers et al., 1979) and the microbicide and cytotoxic activities may be associated with the alkyl formation capacity. Significant inhibition of radicle growth of *Amaranthus hypochondriacus* was reported by Mata et al. (2003). The crude extracts and their fractions are cytotoxic against five human breast cancer cell lines (Molina-Salinas et al., 2006).

## 9. Relevant achievements of *F. cernua* research

The crude extracts and fractions of different polarity inhibit mycelial development of *Rhizoctonia solani*, *Pythium* sp., *Fusarium oxysporum*, *Colletotrichum fragariae*, *Colletotrichum*

*gloeosporioides* Penz, *Colletotrichum accutatum* Simmons, *Phytophthora infestans*, *Alternaria* sp., *Alternaria alternata*, *Penicillium digitatum*.

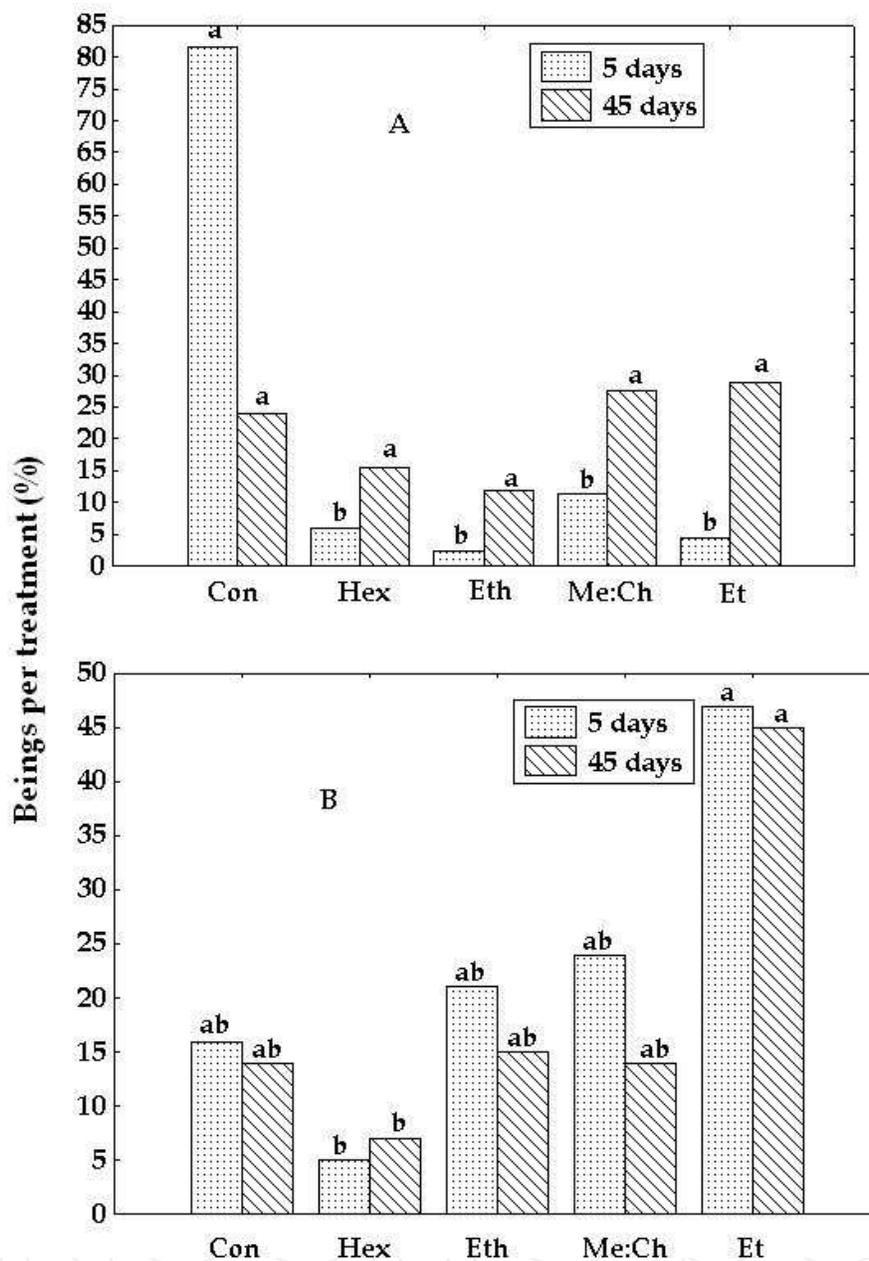


Fig. 6. Percentage of beings found in maize grains treated with four extracts of *Flourensia cernua* at 20,000  $\mu\text{l L}^{-1}$  in (A) raffia sacks and (B) jute sacks. Con=Control; Hex=Hexane; Eth=Ether; Me:Ch=Methanol-Chloroform; Et= Ethanol.

*Flourensia cernua* extracts have a high bioinsecticide activity for controlling *B. brassicae* and insectistatic effect when provoking repellency of *S. oryzae* and high degree of antitermite activity.

The antibacterial activity from the hexane extract of the leaves against *M. tuberculosis* suggests that *F. cernua* could be an important source of non-polar compounds with bactericidal activity.

Crude extracts and its fractions are cytotoxic against five human breast cancer cell lines.

The antioxidant activity of this plant is considered to increase the fungicide activity of the extracts.

The institutional (UAAAN) research results as to the bactericidal and insecticide activity are scientifically reported for the first time.

## 10. Conclusions

The secondary metabolites of semidesert plants as a result of genetic, climatic, and soil factors, vary greatly from plants which develop under less extreme environmental conditions.

*Flourensia cernua* is a Mexican semidesert plant which proved to have the capacity to control *Rhizoctonia solani*, *Pythium* sp., *Fusarium oxysporum*, *Colletotrichum fragariae*, *Colletotrichum gloeosporioides* Penz, *Colletotrichum accutatum* Simmons, *Phytophthora infestans*, *Fusarium oxysporum*, *Alternaria* sp., *Alternaria alternata*, *Penicillium digitatum*, diseases which cause great worldwide losses in field and postharvest production of high commercial value cultivars as tomato, potato, apple, avocado, papaya, banana. As bioinsecticide it also controls the activity of *Brevicoryne brassicae*, which attacks different species of the *Cruciferae* family. Besides, it's a repellent for *Sitophilus oryzae* which attacks stored grains.

The hexane fraction of *F. cernua* leaf mainly contains monoterpenoids, while the ethanol fraction mainly contains sesquiterpenoids, volatile compounds. This extract also contains an unknown number of molecules next to the active principle.

Crude extracts and its fractions of this plant are cytotoxic against five human breast cancer cell lines

Phenolic compounds found in extracts could be responsible of this species antioxidant activity, besides, these extracts don't show cytotoxicity.

*Flourensia cernua* phenolic compounds may be used as food additives with the purpose of preventing oxidation and in general, granting health benefits.

Taking into account the biological importance of this plant compounds, average and long term research must continue focusing on the following: 1. Isolation and identification of the plant active compounds; 2. *In vivo* evaluation of isolated extracts and compounds activity; 3. Product formulation for its industrial and pharmacological use.

Experimental results show a good correlation with the use of this plant in traditional Mexican medicine.

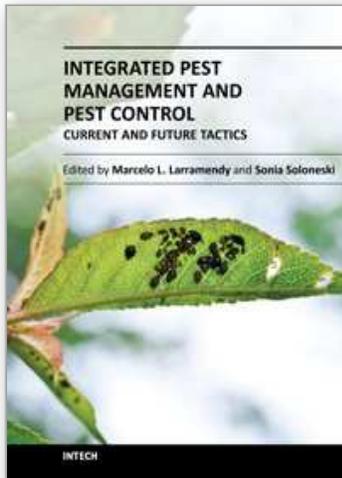
Because *F. cernua* is an endemic species, in the future it's necessary to carry out a domestication for the commercial production of this species to be able to have enough vegetal material for its industrialization and commercialization.

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Integrated Pest Management is an effective and environmentally sensitive approach that relies on a combination of common-sense practices. Its programs use current and comprehensive information on the life cycles of pests and their interactions with the environment. This information, in combination with available pest control methods, is used to manage pest damage by the most economical means and with the least possible hazard to people, property, and the environment.

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