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Evaluation of Resin Content and the Antifungal Effect of *Larrea tridentata* (Sesse and Moc. Ex D.C.) Coville Extracts From two Mexican Deserts Against *Pythium* sp. Pringsh.

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**Abstract.** The antifungal effect of *Larrea tridentata* extracts, a plant locally known as “gobernadora or creosote bush” from northern Mexico, was investigated using fungal inhibition bioassays at 0, 500, 1000, 2000, 4000, and 8000 µl l⁻¹. Samples of *L. tridentata* were collected from Coahuila and Zacatecas states in the Chihuahuan Desert (ChD), and from South Baja California state in the Sonoran Desert (SD). Resin from leaves and small twigs was extracted with methanol, ethanol, and chloroform. Results showed that the overall resin mean value for samples collected in the ChD was 22.6% whereas, those from the SD was 25.49%. Mycelial growth of the phytopathogen *Pythium* sp. was completely inhibited with the extracts evaluated. The fungicidal effect of gobernadora extracts was consistent in spite of the solvent used for extraction or location of sampling sites. Methanolic extracts from both deserts had a notable effect at relatively low doses (800 µl l⁻¹), where fungal growth in vitro did not occur. The overall results indicate that resin extracts from the SD were superior in its inhibitory effect than those from the ChD. *L. tridentata* extracts could be of practical use as antifungal compounds with low impact on the environment with important industrial possibilities. Additional studies under greenhouse and field conditions are required, before the development of new agricultural based organic fungicides and other *Larrea*-based agrochemicals.

**Resumen.** El efecto antifúngico de extractos de *Larrea tridentata*, planta localmente conocida como “gobernadora o arbusto de creosota” del norte de México fue investigado mediante bioensayos inhibitorios para hongos, a dosis de 0, 500, 1000, 2000, 4000 y 8000 µl l⁻¹. Muestras de *L. tridentata* se colectaron en los estados de Coahuila y Zacatecas del Desierto Chihuahuense (DCh) y en Baja California Sur del Desierto Sonorenses (DS). La resina de hojas y ramas pequeñas fue extraída con metanol, etanol y cloroformo. Los resultados mostraron que el valor promedio de resina de las muestras colectadas en el DCh fue de 22.6%, mientras que las del DS resultaron de 25.49%. El crecimiento micelial del hongo fitopatógeno *Pythium* sp. fue completamente inhibido con los extractos evaluados. El efecto fungicida de los extractos de gobernadora se mostró consistente, independientemente del solvente usado para la extracción o del sitio geográfico de colecta. Los extractos metanólicos de ambos desiertos tuvieron un notable efecto a dosis relativamente bajas (500 µl l⁻¹), ya que no hubo crecimiento del hongo in vitro. Los resultados indican que los extractos de resina del DS fueron superiores en su efecto inhibitorio que los del DCh. Los extractos de *L. tridentata* pueden ser de uso práctico como compuestos antifúngicos de bajo impacto ambiental y con importantes posibilidades industriales. Se requieren estudios adicionales bajo condiciones de invernadero y campo, antes de desarrollar nuevos fungicidas y otros agroquímicos con base en extractos de *Larrea*.

Palabras clave adicionales: Gobernadora, creosote bush, organic fungicide, fungicidal activity.
Larrea tridentata (Seese and Moc. ex D.C.) Coville, commonly named “gobernadora or creosote bush” is a xerophytic perennial evergreen shrub, whose individual age probably exceeds 100 years, although some clumps may survive hundreds or thousands of years (Brinker, 1993). L. tridentata is ecologically dominant and widely distributed in the semi-arid regions of North America extending from southern United States to northern Mexico (Downum et al., 1988). The leaves of this xerophytic shrub are covered with a resinous coating that contains a complex mixture of phenolics, saponins, terpenoids and wax esters that account for 10-20% of the leaf dry weight (Mabry et al., 1977; Seigler et al., 1974). Greenfield et al. (1987) reported that between neighboring shrubs there are significant variations in resin content. Over 80% of the resin is composed of phenolic aglycones with the major component being nordihydroguaiaretic acid (NDGA); this catechol lignan is a potent antioxidant and mediates important biocidal effects (Gnabre et al., 1995; Vargas-Arispuro et al., 1997; Verástegui et al., 1996). There is a difference in mean NDGA concentration among Larrea ploidy races depending on the geographical area where gobernadora evolved; shrubs from the Chihuahuan desert report 2.62%, while those from the Sonoran desert contain 3.84% (Gisvold, 1948). On the other hand, the phytopathogenic fungus Pythium spp. Fringsh. attacks a great number of crops, both annual and perennial, infecting plants in all growth stages, but it is most conspicuous as seed or seedling pathogen, and it is among the most widespread root pathogens (Wilhelm, 1984). Pythium spp. cause a pre-emergence and post-emergence seedling blight or damping-off; in some parts of the United States, it is the pathogen most frequently isolated from germinated, but nonemerged diseased seedlings (DeVay et al., 1977). In pepper nurseries, this fungus is responsible for the most troublesome damage to seedlings (Nuez et al., 1996). Control of this disease is primarily dependent upon continued applications of conventional synthetic fungicides, although effective, their continued or repeated use for several decades has brought undesirable effects on the environment and human health concerns. To minimize the adverse effects of synthetic products on agro-ecosystems and emergence of phytopatogens resistant to the currently used fungicides, it is necessary to evaluate other alternatives to control this pathogen (Kim et al., 1999; Russell et al., 1995; Tanaka and Omura, 1993). Therefore, the aim of this study was to evaluate the resin concentration extracted by three solvents, and to investigate the fungicidal effect of L. tridentata extracts from two desertic regions of northern Mexico, on the in vitro inhibition of Pythium sp.

MATERIALS AND METHODS

Area of sampling. L. tridentata extracts were obtained from approximately 10 kg of gobernadora foliage collected from the Chihuahuan desert (ChD) located in the states of Zacatecas and Coahuila in 2000, during November and December, from four different sites (west of Paseo de San Juan, Guadalupe Victoria, Las Esperanzas, and Monclova). Other samples were also collected from four sites of South Baja California Sonoran desert (SD) (north of San Pedro, Loreto, Ciudad Constitución and south of Mulegé). Sampling areas were located at similar latitudes (24, 25, 26, and 27°).

Larrea leaf resin content. Foliage samples were dried in an oven at 65°C for five days to constant weight. The resin determination for each sampling site was done independently by triplicate with methanol, ethanol, and chloroform analytical grade (ACS regent), with a Soxhlet extraction equipment (ASTM, 1993), placing 10 g of dry leaves in a extraction thimble and 250 ml of each solvent. The solvent was brought to the boiling point during 8-10 h, and the pure solvent vapors produced a drip into the thimble. Resin content of leaves and small twigs was calculated according to the following equation: Rc = W2 / W1 *100; where: Rc = Resin content (%); W1 = weight of sample prior to extraction (g); and W2 = weight of sample after extraction (g).

Extract preparation. In order to produce enough resin for the assays, L. tridentata leaves from each sampling site were submersed into a 20 L container separately with each one of the three solvents already mentioned, during 24 h at room temperature; the resulting product was separated from the foliage with a cheesecloth, vacuum filtered through Watham paper No. 1, and the solvent evaporated. After this procedure, the resin was treated with a 3% alkaline solution based on sodium hydroxide, and evaporated to dryness in an oven at 65°C during 5 to 7 days to obtain the solid extracts, that later

<table>
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<th>Solvent</th>
<th>ChD</th>
<th>SD</th>
<th>ChD</th>
<th>SD</th>
<th>ChD</th>
<th>SD</th>
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Table 1. Mean resin concentration extracted with three solvents from Larrea tridentata leaves collected in the Chihuahuan and Sonoran deserts of Mexico.

MeOH = Methanol; EtOH = ethanol; CHCl3 = chloroform.

ChD = Chihuahuan Desert; SD = Sonoran Desert. Resin values are the mean of three extractions performed with Soxhlet equipment from leaves collected at each sampling site in both deserts.
were ground in a mortar and pestle to obtain a hydrosoluble powder.

Bioassay. To study the inhibitory effect of extracts, bioassays were carried out in Petri dishes. *L. tridentata* hydrosoluble extracts dissolved in distilled water were amended to potato-dextrose-agar (PDA) (Bioxon-Becton Dickinson) growing medium (approximately 20 ml/plate). A small portion of an agar disc containing the fungus was placed at the center of each plate, and incubated in a growing chamber for 240 h at 25°C. At the end of this incubation period, the mycelial growth was measured with the aid of a vernier.

Inoculum. *Pythium* sp. was isolated from partially diseased pepper plant tissue by surface disinfecting pieces of infected parts showing disease symptoms. The oospores were produced in V-8 juice broth (20% Campbell’s V-8 juice plus 4.5 g of CaCO₃ per liter of deionized water (Mitchell and Rayside, 1971)).

Experimental design. The experiment was analyzed as a 2 x 4 x 7 factorial with four replications. The A factor was the Sonoran and Chihuahuan deserts; B, the four sampling sites from each desert; and C, the dosage tested (0, 500, 1000, 2000, 4000, 6000 and 8000 µl l⁻¹). Means separation were calculated by LSD (P < 0.01).

**RESULTS AND DISCUSIÓN**

*L. tridentata* leaf resin content. Resin content extracted with the three solvents and collected in two desertic zones ranged from 15.20 to 37.43% (Table 1). These values are higher than those reported by Rhoades (1977) 10 to 26%, however, in that study *L. tridentata* from the Arizona populations was treated with diethyl ether as an extraction solvent. In other studies, Duisberg (1951) obtained leaf resin values of 30-35% extracted with 95% EtOH. These values were similar to those obtained with methanol from the Chihuahuan (ChD) and Sonoran (SD) deserts. Independently of the solvent used for resin extraction, we found that the overall resin mean values of samples collected in the SD were slightly higher (2.89%) than those obtained from ChD. Mean values for resin concentration from ChD samples extracted with the three solvents, presented noticeable differences among the collection sites, but the pattern was inconsistent along the geographic range of the populations sampled (Fig. 1). Meanwhile, resin concentration from SD samples showed little, but a consistent variation from southern to northern sampling sites, being higher at 24° (26.89%) and lower at 27° (24.36%). In relation to the solvents used for extraction, mean resin concentration from the SD samples extracted with MeOH were 12.20% and 20.13% higher than the amount of resin obtained with EtOH and CHCl₃, respectively. Similarly, *L. tridentata* resin from ChD samples extracted with MeOH was 4.77% and 7.42% higher than the amounts extracted with EtOH and CHCl₃, respectively (Table 1). The values obtained in this research could be an indication of differences due to the polarity of the solvent used (Chang, 1998), the age of plant tissue, with greater resin and NDGA concentration in young plants and leaves (Gravold and Thaker, 1974; Mabry et al., 1977), and/or quantitative phytochemical differences between the diploid populations, characteristic of ChD and the tetraploid populations of SD (Downum et al., 1988; Yang, 1970).

**Antifungal activity of *L. tridentata* extracts.** Methanol extracts dramatically inhibited *in vitro* mycelial growth of the fungus; this effect was more evident because even at the lowest dose evaluated (500 µl l⁻¹), resin extracts from both deserts completely inhibited fungal growth (Fig. 2A); at higher doses, MeOH extracts from all collection sites (parallels) totally inhibited growth of *Pythium* sp. Similar fungicidal activity of crude resin extracts had been reported by Hurtado et al. (1979), however these authors did not find a complete inhibition of this fungus even at the highest dose (1000 µl l⁻¹) studied. Similarly, Vargas-Arispuro et al. (1997) demonstrated a fungistatic activity of *L. tridentata* methanolic extracts, however, they were less effective against *Aspergillus flavus* Link:Fr. and A. parasiticus Speare. A clear inhibitory effect was observed with EtOH extracts at 500 and 1000 µl l⁻¹ (Fig. 2B), at the lowest concentration a fungistatic effect was observed in all geographic sampling sites, while for the second concentration this effect was observed only at the sites located at the south latitude (parallel 24°). Varástegui et al. (1996), found that *L. tridentata* resin extracted with 80% ethanol also inhibited the *in vitro* growth of *Listeria monocytogenes* (Murray, Webb and Swann) Pirie, *Clostridium perfringens* (Veillon and Zubér) Hauduroy, Ehringer, Urbain, Guillot and Magrou, *Shigella dysenteriae* (Shiga) Castellani and Chalmers, *Versinia enterococaltica* (Schleffstein and Coleman) Frederiksen, *Proteus vulgaris* Hauser, *Nocardia asteroides* (Eppinger) Blanchard, and *N. brasiliensis* (Lambergen) Pinoy. In regard with the solvents used for resin extraction, mean values of the extracts inhibitory effect on mycelial growth, indicate that only at 500, 1000, and 2000 µl l⁻¹ concentrations,

![Fig. 1. Mean resin concentration extracted with three solvents of *Loreo tridentata* foliage, collected from four sampling sites (parallels) in the Sonoran and Chihuahuan deserts from northern Mexico.](image-url)
Our data indicate that Sonoran desert extracts showed greater overall inhibitory effect (85.07%) than 80.92% found with the Chihuahuan extracts (Table 2). Significant differences in the fungus inhibitory effect among sampling areas from the two deserts were found, since SD extracts from parallel 26° were the most effective by inhibiting 86.79% the in vitro mycelial growth of *Pythium* sp.; meanwhile, resins from parallel 25° reported the lowest (81.20%) inhibitory effect of the SD extracts. Compared to the other sampling areas, extracts from parallel 25° of ChD showed the lowest inhibitory effect. However, it was observed an excellent fungitoxic effect on mycelial growth at the remainder concentrations. After finishing the bioassays for each extract, *Pythium* inoculum was transferred to fresh medium, where the fungus did not grow, indicating that the effect of same treatments was rather fungicidal than fungistatic.

**CONCLUSIONS**

The higher leaf resin concentration from native stands of *Larrea tridentata* plants were obtained from samples collected in the Sonoran Desert. The slightly greater fungicidal effect on *Pythium* sp. detected with extracts from the Sonoran desert compared to extracts from the Chihuahuan desert, could be attributed to higher leaf resin content and consequently more compounds with fungicidal properties. The efficacy of hydro soluble laboratory-prepared extracts from *L. tridentata* leaves tested against *Pythium* sp. demonstrated their fungicidal properties for in vitro studies. Methanolic extracts showed better fungicidal effect than ethanolic and

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<th>Deserts / Parallels</th>
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<tr>
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<td><strong>Chihuahuan desert</strong></td>
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<td>80.92</td>
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1 Sampling areas were located at similar latitudes on both deserts.
2 Percentage inhibition values correspond to the mean value of six doses evaluated and the three solvents used for *L. tridentata* resin extraction.
3 Mean values followed by the same letter are not significantly different (p < 0.01). LSD = 4.1860.
chloroformic extracts. To our knowledge, this is the first report showing a clear fungicide effect of *L. tridentata* extracts on *Pythium* sp. growth at 500 µl L⁻¹ concentration. Our results support the effectiveness of antifungal compounds from gobernadora or creosote bush for *in vitro* studies. For the convenient use of hydrosoluble extracts as commercial fungicides, they have to be further evaluated on *in situ* studies; greenhouse and field conditions will be required in future work.

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LITERATURE CITED


