REVISTA CHAPINGO SERIE HORTICULTURA
ISSN: 1027-152X
revistahorticultura29@gmail.com
Universidad Autónoma Chapingo
México

Lira-Saldivar, R. H.; Hernández-Suárez, M.; Hernández-Castillo, F. D.
Activity of Larrea tridentata (D.C.) Coville L. extracts and chitosan against fungi that affect horticultural crops
REVISTA CHAPINGO SERIE HORTICULTURA, vol. 12, núm. 2, julio-diciembre, 2006, pp. 211-216
Universidad Autónoma Chapingo
Chapingo, México

ACTIVITY OF *Larrea tridentata* (D.C.) Coville L. EXTRACTS AND CHITOSAN AGAINST FUNGI THAT AFFECT HORTICULTURAL CROPS

R. H. Lira-Saldivar[1¶]; M. Hernández-Suárez[1]; F. D. Hernández-Castillo[2]

[1]Centro de Investigación en Química Aplicada. Blvd. Enrique Reyna Núm. 140, Saltillo, Coahuila. C. P. 25253, México. Tel/Fax (844) 438-9830. E-mail: rhlira@ciqa.mx ([¶]Corresponding autor).

[2]Universidad Autónoma Agraria Antonio Narro, Departamento de Parasitología Agrícola, Buenavista, Saltillo, Coahuila C. P. 23315, MÉXICO.

SUMMARY

The antifungal activity of gobernadora [*Larrea tridentata* (D.C.) Coville (L.)] hydrosoluble resin extract and chitosan solutions (Ch), alone and in combination, were investigated *in vitro* for their bioactivity against the fungi *Botrytis cinerea*, *Colletotrichum coccodes* and *Fusarium oxysporum* f. sp. *lycopersici* that were isolated from greenhouse roses, potatoes and tomatoes from commercial plots, respectively, and later purified. Both bioproducts demonstrated their fungicidal effect at 1000 and 2000 ì l·liter^{-1}; however, when they were combined they exhibited synergistic fungicidal activity. These preliminary results indicate that *L. tridentata* hydrosoluble resin extract or the combination L-Ch could be considered as potential agrochemicals of low environmental impact for use as organic fungicides; however, further work is needed before this application could be used commercially. To the best of our knowledge, this is the first time that a mixture of *L. tridentata* and chitosan has been reported acting as antifungal compound.

ADDITIONAL KEY WORDS: organic fungicides, biopolymers, creosote bush, plant extracts, synergistic effect.

ACTIVIDAD DE EXTRACTOS DE *Larrea tridentata* (D.C.) Coville (L.) Y QUITOSÁN CONTRA HONGOS QUE AFECTAN CULTIVOS HORTÍCOLAS

RESUMEN

La actividad antifúngica de extracto de resina hidrosoluble de gobernadora [*Larrea tridentata* (D.C.) Coville (L)] y soluciones de quitosán (Ch), solos y combinados fueron investigados *in vitro* por su actividad antifúngica contra *Botrytis cinerea*, *Colletotrichum coccodes* y *Fusarium oxysporum* f. sp. *lycopersici* que fueron aislados de rosas de invernadero y de lotes comerciales de papa y tomate, respectivamente, mismos que posteriormente fueron purificados. Ambos bioproductos manifestaron su efecto funguicida a 1,000 y 2,000 ì l·litro^{-1}, sin embargo, cuando fueron combinados mostraron una actividad fungicida sinérgica. Estos resultados preliminares indican que el extracto hidrosoluble de *L. tridentata* o la combinación L-Ch pudiesen ser considerados como agroquímicos potenciales de bajo impacto ambiental para ser usados como fungicidas orgánicos, pero se requiere más trabajo de investigación antes de que esto tenga una aplicación comercial. Hasta lo mejor de nuestro conocimiento esta es la primera vez que se reporta a la mezcla *L. tridentata* y quitosán actuando como un compuesto antifúngico.

PALABRAS CLAVE ADICIONALES: fungicidas orgánicos, biopolímeros, gobernadora, extractos vegetales efecto sinérgico.

INTRODUCTION

Botrytis cinerea Pers. is considered an omnipresent pathogen around the world that causes gray mold on a large number of economically important crops during the growing season and throughout post harvest (Keller *et al*., 2003); also, it is the most common disease of glasshouse crops (Daugherty *et al*., 1995). Fungal diseases like black dot of potatoes, caused by *Colletotrichum coccodes* (Wallr.) S. J. Hughes, was considered a minor disease worldwide until the early 1990s, when reports from several countries indicated increasing occurrences of the disease and greater impacts on potato production (Tsror *et al*., 1999). This disease is now being found in certain potato growing areas of Mexico; it causes aerial infections, premature plant death, and, as a soil-borne pathogen, it can interact with other fungi

Recibido: 25 de mayo, 2006
Aceptado: 26 de septiembre, 2006

to increase symptom expression and yield reduction on potato (Lees and Hilton, 2003). One of the worldwide phytosanitary problems limiting tomato production is vascular wilting caused by *Fusarium oxysporum* f. sp. *lycopersici* Snyder and Hansen, which occurs more in warm weather where it causes great economic losses (Carrillo-Fasio *et al*., 2003). This fungus causes severe crown and root rot of tomato, as well as, extensive necrotic lesions in the basal portion of the stem, wilting, and plant death. *Fusarium oxysporum* f. sp. *radicis-lycopersici* is another very damaging fungus that has reduced yield of tomato up to 50 % in the state of Sinaloa (Apodaca-Sanchez *et al*., 2002), which is the most important tomato producing region of Mexico. Sanitation, cultural practices and chemical fungicides are used as control measures against *B. cinerea*, *C. coccodes*, and *F. oyxysporum* in Mexico and elsewhere, but growers depend chiefly on synthetic fungicides (Romanazzi *et al*., 2002; Lees and Hilton, 2003). Although effective, their continued or repeated use for several decades has disrupted natural control and has lead to outbreaks of diseases, undesirable effects on no target organisms, and environmental and human health concerns.

Botanical product-based pesticides offer advantages in that they can sometimes be specific to the target species and typically have unique modes of action with little mammalian toxicity. Furthermore, they generally do not persist in the environment (Duke *et al*., 2003). Creosote bush or gobernadora [*Larrea tridentata* (D.C.) Coville (L.)] is the most abundant shrub on millions of hectares of the North American warm deserts (Chihuahuan, Sonoran and Mohave). The leaves of this shrub are covered with a resinous coating that contains a complex mixture of phenolics, saponins, terpenoids and wax esters that account for 20 - 35 % of the leaf dry weight (Table 1). Over 80 % of *Larrea* resin is composed of phenolic aglycones with the major component being nordihydroguaiaretic acid (Brinker, 1993), this catechol lignan is a potent antioxidant and mediates important biocidal effects in diverse microorganisms (Gnabre *et al*., 1995). A notable phytochemical attribute of gobernadora shrubs is that they produce a thick and water insoluble resin that accumulates in leaves and small twigs. The resin has shown to have fungicidal and bactericidal properties in many pathogens (Verástegui *et al*., 1996; Lira-Saldivar *et al*., 2002; Lira-Saldivar *et al*., 2003a). At present, an increasing interest is being devoted to the use of natural substances such as chitosan a biopolymer derived from chitin which is very similar to cellulose (Figure 1). This high molecular weight cationic polysaccharide that occurs in fungal cells walls and arthropods exoskeletons had been considered as a valid alternative to synthetic fungicides (Romanazzi *et al*., 2003). Indeed, chitosan is an ideal preservative coating for fresh fruit and vegetables because of its film-forming and biochemical properties (Muzzarelli, 1986). Also, it is reported to prolong storage life and control decay of several fruits (Romanazzi *et al*., 2002). Therefore, the objective of this research was to find out the amount of leaf resin concentration from different native populations of *L. tridentata* shrubs

TABLE 1. Main phytochemical constituents of *Larrea tridentata* (Brinker, 1993).

Dry weight (%)	Class/type	Compound
16 - 21	Phenolics/ lignans	Dihydroguaiaretic acid
		Hemi-norisoguaiacin
		Nordihydroguaiaretic acid
		Norisoguaiacin
5 - 7.5	Flavonoids/aglycone	Apigenin
		Kaempferol
	Flavonoids/glycosides	Chrysoeriol
		Quercetin
10 - 15	Saponins/triterpenes	Larreagenin A
		Larreic acid
70.1 (of stems)	Lipids/wax esters	Alkyl esters

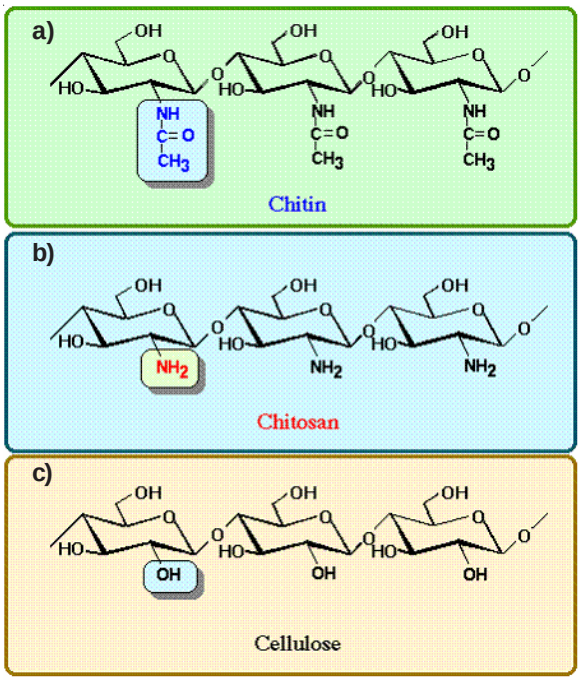

FIGURE 1. Chemical structures of the biopolymers cellulose (a), chitin (b), and its derivative chitosan (c).

located in northern Mexico, and whether *Larrea* extract and chitosan, alone and combined, could prove to be effective as antifungal agents, and lastly to determine if mixtures of both biocompounds can have a synergistic effect against three fungi that have a harmful effect on many horticultural crops.

MATERIALS AND METHODS

Sampling sites and climatic characteristic

L. tridentata foliage for the resin extracts was collected

during March 2004 from a zone of the Chihuahuan Desert located in the states of Zacatecas and Coahuila. Four places (San Tiburcio, La Pardita, Pabellón Hidalgo and Concepción del Oro) were situated in Zacatecas, and another four (Encarnación de Guzmán, Gómez Farías, Tanque de Emergencia and Agua Nueva) in Coahuila. All sites were located between the geographic coordinates 24° and 25 ° N and 101° and 102° W, at an altitude range from 2,200 to 2,600 m. According to the classification of Köppen, modified by García (1988) the climate of this region is type BWw (x') h (e), which is very arid with scarce and torrential rains during the long summer and cold temperatures during the winter.

L. tridentata resin content and chitosan preparation

Foliage samples were dried in an oven at 65 °C for five days to constant weight. Resin determination for each sampling site was done independently by triplicate with ethanol analytical grade (ACS regent), with a Soxhlet extraction equipment (ASTM, 1993), placing 10 g of dry leaves in an extraction thimble and 250 ml of methanol as the solvent. The solvent was brought to a boiling point for 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). In order to produce enough resin for the assays, *L*. *tridentata* leaves from each sampling site were submerged during 24 h at room temperature into a 20-liter container separately with the organic solvent already mentioned; the resulting product was separated from the foliage with cheesecloth, vacuum filtered through Waltham No. 1 paper and the solvent evaporated in a rotary evaporator. Crab-shell chitosan, purchased from Sigma Aldrich Co. (Sheboygan WI, USA), was arranged as described by El Ghaouth *et al*. (1992). For experimental use the stock solution, (1.0 %, w/v) was prepared by dissolving purified chitosan in 0.5 % (v/v) glacial acetic acid under continuous stirring during 16 h, and the pH was adjusted to 5.6 using 1 N NaOH (Romanazzi *et al*., 2003). The stock solution was autoclaved and appropriate concentrations were obtained by dilution with deionized distilled water.

Isolation and identification of plant pathogens

B. *cinerea* was isolated from diseased greenhouse roses (*Rosa* spp.); *C*. *coccodes* from diseased potatoes (*Solanum tuberosum* L.) and *F. oxysporum* from diseased tomatoes (*Lycopersicon esculentum*) obtained both from commercial plots. To isolate the fungi from vegetative material, pieces of infected tissue were taken and disinfested with sodium hypochlorite at 1.5 % during 5 min and later were planted in growth medium potato dextrose agar (PDA) and incubated at 25 °C. In order to carry out fungal identification, sporulation was induced under continuous artificial light; once the fungi growth fully covered Petri dishes and spores were produced, microscopic preparations were made with blue lactophenol, and with the aid of a microscope the fungi were identified using the key of Barnett and Hunter (1998).

Detection of antifungal activity in semi-solid medium

To study the inhibitory effects of the bioproducts, assays were performed with Petri dishes in which dissolved *L*. *tridentata* (L) extract and chitosan (Ch) were added alone or mixed to potato dextrose agar (PDA) growth medium (Bioxon; Becton Dickinson of Mexico). Chitosan solutions and PDA were autoclaved separately and combined after autoclaving. Equal volumes of acid were used for all concentrations of chitosan, adjusted to pH 5.6 to keep the salt concentration constant. Eleven treatments were evaluated with each plant pathogen: three L doses (1,000, 2,000 and 4,000 ì l·liter^{-1}), two Ch concentrations (1,000 and 2,000 ì l·liter^{-1}), four L-Ch mixtures (1,000-1,000; 2,000-1,000; 1,000-2,000 and 2,000-2,000 ì l·liter^{-1}) plus two controls: (i) distilled water (dw) and (ii) a synthetic fungicide (Chlorotalonil and Prozycar; 2000 ì l·liter^{-1}). After thorough mixing, the molten medium was dispensed into 9-cm diameter Petri dishes (approximately 20 ml·plate^{-1}). *In vitro* experiments were carried out using 7-day-old cultures of *B*. *cinerea*, 10-day-old for *C*. *coccodes*, and 13-day-old for *F*. *oxysporum*. Mycelia plugs of each of the fungi were placed at plate centers and incubated at 25 °C during 5, 20 and 14 days for each fungi, respectively; at the end of the incubation period growth of each pathogen was determined by measuring fungal radial growth with the aid of a vernier. Percent inhibition (PI) was determined by using the following equation: PI = 100 – [(Mrg * 100) / Pr]; in which Mrg = mycelia radial growth (mm) and Pr = plate radius (mm). Spore counts per ml of each fungus were performed with a Neubauer hematocytometer using a microscope with the 40 X objective. A completely randomized experimental design with six replicates was used for the resulting treatments. In order to determine whether a synergistic interaction was produced by the interaction between *L*. *tridentata* and chitosan treatments, Limpel's method as described by Romanazzi *et al*. (2003) was used. Limpel's formula is E_e = X+Y-(XY/100), in which E_e is the expected effect from additive responses of two inhibitory agents, and X and Y are the percentages of inhibition relative to each agent used alone. Thus, if the combination of the two agents produces any value of inhibition greater than E_e, then synergism exists.

RESULTS AND DISCUSSION

Leaf resin content

L. tridentata resin extracted with methanol ranged from 19.45 to 31.23 %, with a mean value of 21.75 and 26.30 %, respectively, for the Zacatecas and Coahuila populations (Table 2). Our *L. tridentata* leaf resin content values reported here are higher than 10 to 26 % extracted with diethyl ether as an extraction solvent reported by Rhoades (1977) from Arizona populations of *L. tridentata*. A former study reported by Lira-Saldivar *et al*. (2003b) pointed out that mean leaf resin content extracted with methanol and ethanol ranged from 36.27 to 34.43 %, respectively for *Larrea* populations belonging to the Chihuahuan and Sonoran deserts. This difference among resin concentrations could be attributed to

the higher polarity and extractive power of methanol as a solvent; and also to the fact that foliage was collected after the rainy season, when the production of secondary metabolites was at the highest point. Place-to-place variations in foliage resin content is normally related to a variety of abiotic and biotic factors such as levels of soil moisture, soil slope, air temperature; leaf development, plant age and leaf maturation (Lira-Saldivar *et al*., 2003a); therefore, the inter- and intrapopulation variation detected for resin content was dependent upon *L. tridentata* populations and ecological characteristics of the sampling sites. In this work we observed that the proportion of resin content follows a general pattern of decrease among populations and along a soil and air humidity gradient from northern Zacatecas to southeastern Coahuila. González-Coloma *et al*. (1988) investigated the impact of ozone concentration on NDGA levels in *Larrea* resin; they also reported a substantial geographic and intrapopulation variation, which in turn depended on abiotic factors such as soil moisture and soil temperature.

Mycelia radial growth inhibition

The effect of *L*. *tridentata* extract, chitosan and combinations of both bioproducts on Mrg varied according to the treatment and fungi. The data shows that the level of inhibition by *L*. *tridentata* and chitosan treatments by themselves were not as great as that of mixtures of both products on *C*. *coccodes* and *F. oxysporum* (Table 3). Mean value for Mrg inhibition was 98.86 % on *B*. *cinerea*, followed by 82.75 % and 65.61 % on *C*. *coccodes* and *F. oxysporum* respectively. The maximum mycelia inhibition (100 %) on *B*. *cinerea* was obtained with L-Ch combination at the concentrations 2000-1000; 1000-2000 and 2000-2000 ì l·liter^{-1}; these treatments significantly (P<0.01) reduced Mrg compared to the control treatment (distilled water), and reported the same antifungal effect as the synthetic chemical control (chlorothalonil 2000 ì l·liter^{-1}); however, *L*. *tridentata* extract and chitosan as separate compounds also reduced to a large extent *B*. *cinerea* growth. *L*. *tridentata* resin extract was effective in inhibiting mycelia growth and sporulation of *B*. *cinerea*, *C*. *coccodes* and *F*. *oxysporum*. Many reports are available that corroborate the effect of *L*. *tridentata* resin extract against numerous fungi and other microorganisms

TABLE 2. Mean values of resin concentration extracted with methanol from *Larrea tridentata* leaves collected from four sites in a portion of the Chihuahuan Desert.

Leaf resin concentration (%) by location			
Zacatecas State		**Coahuila State**	
San Tiburcio	19.45[z]	Encarnación de Guzmán	22.67
La Pardita	23.68	Gómez Farías	26.96
Pabellón Hidalgo	21.34	Tanque de Emergencia	24.35
Concepción del Oro	22.54	Agua Nueva	31.23
Mean	21.75	Mean	26.30

[z]Values are the mean of three replicates from leaves collected at each sampling site in the states of Zacatecas and Coahuila, Mexico.

TABLE 3. Mycelia growth inhibition of *Botrytis cinerea*, *Colletotrichum coccodes* and *Fusarium oxysporum* after the incubation period on dishes containing different concentrations of *Larrea tridentata* (L) and chitosan (Ch) extracts, and mixtures of both bioproducts.

	Mycelia growth inhibition (%)		
Treatments (ì l·liter^{-1})	***B. cinerea***	***C. coccodes***	***F. oxysporum***
Control (Distilled H_2O)	0 d[z]	0 f	0 d
Chemical control[y]	100 a	100 a	81.3 b
L-Ch 1000-1000	98.7 a	98.6 a	85.1 b
L-Ch 2000-1000	100 a	99.0 a	96.4 a
L-Ch 1000-2000	100 a	100 a	100 a
L-Ch 2000-2000	100 a	100 a	100 a
L 1000	96.0 c	80.2 b	63.2 c
L 2000	95.9 c	72.2 c	49.1 d
L 4000	99.5 a	64.5 d	41.8 d
Ch 1000	98.3 a	11.4 e	21.0 e
Ch 2000	100 a	100 a	21.8 e
Mean inbibition	98.8	82.6	66.0
LSD	0.47	3.6	9.9
C.V. (%)	4.21	4.42	4.13

[y]Chemical controls used were chlorotalonil and prozycar at 2000 ì l·liter^{-1}
[z]Numbers followed by the same letter do not differ significantly according to Tukey test ($P \leq 0.01$)

(Lira-Saldivar, 2003). On the other hand, chitosan coating proved to be almost as effective as the fungicide thiabendazole in controlling strawberries (*Fragaria* x *ananassa* Duch.) decay caused by *B*. *cinerea* and *Rhizopus* sp.; also, covering with chitosan had beneficial effects on firmness, titratable acidity, vitamin C content, and anthocyanin concentration in strawberries and raspberries (*Rubus idaeus* L.) (Zhang and Quantick, 1998). Likewise, Benhamou *et al*. (1994) reported that coating seed with chitosan resulted in less seedling disease for tomato. A combination of seed treatment plus a soil amendment with chitosan was found to be more effective in protecting tomato seedlings against *F. oxysporum* attack. In the same way, El Ghaouth *et al*. (1992) demonstrated that growing cucumber (*Cucumis melo* L.) plants in the presence of chitosan controlled root rot caused by *Pyhtium aphanidermatum* and triggered several host defense responses, including induction of structural barriers in root tissues and the stimulation of antifungal hydrolases.

Fungi sporulation inhibition

B. *cinerea* and *C*. *coccodes* sporulation was effectively reduced by all treatments. *Botrytis* sporulation did not show statistical differences among the several combinations L-Ch, the same was true for *Larrea* extract and chitosan doses compared to the chemical control, since all treatments had the same inhibitory effect on spore production (Table 4). For *C*. *coccodes* sporulation also decreased steadily from most treatments. The mixtures L-Ch 2,000-1,000, 1,000-2,000 and 2,000-2,000, as well Ch at 2,000 ì l·liter^{-1} totally inhibited spore production of this fungus, the same result was ob-

served with the fungicide chlorothalonil. When *L. tridentata* extract was applied separately, sporulation was partially diminished; therefore, compared to the distilled water control no statistical differences were experienced due to *Larrea* extract. On *F. oxysporum* the combinations L-Ch 2,000-1,000 and 2,000-2,000 ì l·liter^{-1} showed a total inhibitory effect on sporulation. It is noteworthy that these treatments, including *L. tridentata* extract at 4,000 ì l·liter^{-1}, resulted in statistically superior fungitoxic activity (P<0.01) than the chemical control, since chlorothalonil only moderately affected pathogen sporulation. Few commercial fungicides have been effective in inhibiting teliospore germination of *Tilletia indica*, the causal agent of Karnal bunt of wheat (*Triticum aestivum* L.), however, teliospores subjected to the dichloromethane extract from *L. tridentata* showed no viability when transferred to fresh culture media (Rivera-Castañeda *et al*., 2001).

Synergistic effect of *Larrea tridentata* and chitosan mixtures

In order to test the antifungal effect of *L. tridentata* and chitosan, and mixtures of both compounds, innoculi were transferred to fresh medium and the fungi did not grew, indicating that the treatment effect was that of a fungicide. Mycelia inhibition on *C. coccodes* by the combination L-Ch at 1,000-1,000, 2,000-1,000, 1,000-2,000 and 2,000-2,000 ì l·liter^{-1} resulted statistically similar to the effect reported by the chemical control (chlorothalonil at 2000 ì l·liter^{-1}), as well Ch at 2,000 ì l·liter^{-1}. A similar inhibitory pattern to that mentioned before was exhibited by *F. oxysporium*, however, this pathogen was less sensitive to *Larrea* extract and chitosan alone, nevertheless, the combinations L-Ch at 1,000-2,000 and 2,000-2,000 ì l·liter^{-1}, showed total Mrg inhibition, furthermore, statistically superior (P<0.01) to that reported by the chemical control (81.3 %). On the basis of these results, the superior antifungal effect for all three fungi was obtained with the combination L-Ch 1,000-2,000 and 2,000-2,000 ì l·liter^{-1}, since at these concentrations L-Ch formulations resulted in less fungal growth (Table 3) than the expected additive response, as determined by calculating the E_e values according to Limpel's formula (Lorito *et al*., 1994). The E_e value for percentage reduction of total mycelia growth on *C. coccodes* using 2,000 ì l·liter^{-1} *L. tridentata* extract and 1,000 ì l·liter^{-1} chitosan and its combination was 72.2 + 11.4 - [(72.2 x 11.4)/100] = 75.37, where the observed inhibition value was 99.0 %. Thus, the combination of 2,000 and 1,000 ì l·liter^{-1} produced a synergistic effect on *C. coccodes* mycelia growth inhibition. The same synergistic effect was observed on *F. oxysporum*, since the combination of L-Ch 1,000-2,000 and 2,000-2,000 ì l·liter^{-1} reported complete (100 %) growth inhibition (Table 3), although *L. tridentata* extract at 1,000 and 2,000 ì l·liter^{-1} reduced fungal growth by 63.2 and 49.1 %, respectively, compared to only 21.8 % for chitosan at 2,000 ì l·liter^{-1}. By applying Limpel's formula we found that E_e values in both cases are 71.23 and 60.19 %, which are lesser than 100 % reported by the combination L-Ch.

TABLE 4. Effect of *Larrea tridentata* (L) and chitosan (Ch) extracts, and mixtures of both bioproducts on sporulation inhibition after the incubation period for *Botrytis cinerea*, *Colletotrichum coccodes* and *Fusarium oxysporum*.

Treatments (ì l·liter^{-1})	Sporulation inhibition x 10^4 ml^{-1}		
	B. cinerea	*C. coccodes*	*F. oxysporum*
Control (Distilled H_2O)	8.3 a[z]	6.7 a	47.7 bcd
Chemical control[y]	0 b	0 a	56.6 bc
L-Ch 1000-1000	0.3 b	0.8 c	102.9 a
L-Ch 2000-1000	0 b	0 c	61.3 b
L-Ch 1000-2000	0 b	0 c	0 e
L-Ch 2000-2000	0 b	0 c	0 e
L 1000	2.2 b	4.2 abc	25.0 bcde
L 2000	1.7 b	6.4 ab	20.3 cde
L 4000	1.7 b	2.8 ab	15.8 de
Ch 1000	0 b	1.7 bc	33.3 bcde
Ch 2000	0 b	0 c	23.0 cde
Mean sporulation	2.8	3.7	42.9
LSD	4.4	4.8	37.4

[y]Chemical control used were chlorotalonil and prozycar at 2000 ì l·liter^{-1}
[z]Numbers followed by the same letter do not differ significantly according to Tukey test (P≤0.01)

The interaction of *L. tridentata*-chitosan at the combinations studied in the present work noticeably increased the antifungal activity compared to the effect of each separate product. Overall, we observed that these mixtures were most effective at 2,000-2,000, 1,000-2,000 and 1,000-1,000 ì l·liter^{-1}, since the results obtained at these concentrations were as effective, and in some cases superior to those exhibited by the synthetic fungicides used as chemical controls (chlorothalonil and prozycar). Thus, the combination of *L. tridentata* resin extract and chitosan produced a synergistic effect in the reduction of mycelia growth and sporulation by *B. cinerea*, *C. coccodes* and *F. oxysporum*. This is in agreement with the work of Romanazzi *et al*. (2003), who showed that the combination of chitosan and short hypobaric treatments provided greater efficiency in controlling grey mold, brown rot, and total rots of sweet cherries caused by *B. cinerea* and other fungi. Lorito *et al*. (1994) also reported that the inhibitory effects on spore germination and germ tube elongation of *B. cinerea*, *F. solani*, and *Uncinula necator* were synergistically increased by mixing fungal enzymes and cells of the bacteria *Enterobacter cloacae*.

CONCLUSIONS

Larrea tridentata plants collected in the southeast part of Coahuila state showed higher leaf resin concentration (22.67 – 31.23 %); however, no noticeably differences were detected regarding its microbial effect on the three fungi evaluated. The antifungal activity of *L. tridentata* and chitosan, alone or in combination, was demonstrated on *B. cinerea*, *C. coccodes* and *F. oxysporum*. The presence of one compound consistently enhanced the effects of the second; there-

fore, we detected a synergistic effect between *L. tridentata* hydrosoluble extract and chitosan solutions. To the best of our knowledge, the synergy linking these biocompounds is reported here for the first time. Our results suggest that the combination L-Ch could be of practical use as antifungal compound for its utilization against diseases that affect many horticultural crops in Mexico and elsewhere. Therefore, the suitable use of these organic products as commercial pesticides requires having them further evaluated through *in vivo* studies under greenhouse and field conditions.

LITERATURE CITED

APODACA-SANCHEZ, M.A.; ZAVALETA-MEJÍA, E.; GARCÍA-ESPINOZA, R.; OSADA-KAWASOE, S. 2002. Frecuencia de campos infestados con *Fusarium oxysporum* f.sp. *radicis-lycopersici* en Sinaloa, México y su control. Revista Mexicana de Fitopatología 20: 1-7.

ASTM (AMERICAN SOCIETY FOR TESTING AND MATERIALS). 1993. G 120-93. Standard test method for determination of soluble residual contamination in materials and components by soxhlet extraction. PA., USA. pp. 1320-1322.

BARNETT, H. L.; HUNTER, B. B. 1998. Illustrated genera of imperfect fungi (Illustrated genera of smut fungi). Burgess Publishing Company, Minnesota. 241 p.

BENHAMOU, N.; LAFONTAINE, P. J.; NICOLE, M. 1994. Induction of systemic resistance to Fusarium crown and root rot in tomato plants by seed treatment with chitosan. Phytopathology 84: 1432-1444.

BRINKER, F. 1993. *Larrea tridentata* (D.C.) Coville (Chaparral or Creosote Bush). British Journal of Phytotherapy 3: 10-30.

CARRILLO-FASIO, J. A.; MONTOYA-RODRÍGUEZ, T. J.; GARCÍA-ESTRADA, R. S.; CRUZ-ORTEGA, J. E.; MÁRQUEZ-ZEQUERA, I.; SAÑUDO-BARAJAS, A. J. 2003. Razas de *Fusarium oxysporum* f. sp. *lycopersici* Snyder y Hansen, en tomate (*Lycopersicum esculentum* Mill.) en el Valle de Culiacán, Sinaloa, México. Revista Mexicana de Fitopatología 21: 123-127.

DAUGHERTY, M. L.; WICK, R. L.; PETERSON, L. 1995. Botrytis blight of flowering potted plants, pp. 11-14. *In*: Compendium of Flowering Potted Plant Diseases. Phytopathological Society Press St. Paul, MN, USA.

DUKE, S.; BAERSON, S. R.; DAYAN, F. E.; RIMANDO, A. M. 2003. United States Department of Agriculture-Agriculture Research Service research on natural products for pest management. Pest Management Science 59:708-717.

EL GHAOUTH, A.; ARUL, J.; GRENIER, J.; ASSELIN, A. 1992. Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. Phytopathology 82: 398-402.

EL GHAOUTH, A. 1997. Biologically-based alternatives to synthetic fungicides for the control of postharvest diseases. Journal of Industrial Microbiology & Technology 19: 160-162.

GARCÍA, E. 1988. Modificaciones al Sistema de clasificación Climática de Köppen, pp. 189-195. Universidad Nacional Autónoma de México.

GNABRE, J. N.; BRADY, J. L.; CLANTON, D. J. 1995. Inhibition of human immunodeficiency virus type 1 transcription and replication by NDA sequence-selective plant lignan. Proceedings of the National Academy of Sciences of the (U. S. A.) pp. 11239-11243.

GONZÁLEZ-COLOMA, A.; WISDOM, CH. RUNDEL, P. 1988. Ozone impact on the antioxidant nordihydroguaiaretic acid content in the external leaf resin of *Larrea tridentata*. Biochemical Systematics and Ecology 16: 59-64.

KELLER, M.; VIRET, O.; COLE, F. M. 2003. *Botrytis cinerea* infection in grape flowers: defense reaction, latency, and disease expression. Phytopathology 93: 316-322.

LEES, A. K.; HILTON, A. J. 2003. Black dot (*Colletotrichum coccodes*): an increasingly important disease of potato. Plant Pathology 52: 3-12.

LIRA-SALDIVAR, R. H.; GAMBOA-ALVARADO, R.; VILLARREAL-CÁRDENAS, L. A.; LÓPEZ-CAMPOS R. G.; JIMÉNEZ-DÍAZ, F. 2002. Hydrosoluble extracts of *Larrea tridentata* from two desertic zones in the north of México and their inhibitory effect on *Fusarium oxysporum*. ÖYTON-International Journal of Experimental Botany 2002: 167-172.

LIRA-SALDIVAR, R. H. 2003. Estado actual del conocimiento sobre las propiedades biocidas de la gobernadora [*Larrea tridentata* (D.C.) Coville]. Revista Mexicana de Fitopatología 21: 214-222.

LIRA-SALDIVAR, R. H.; SÁNCHEZ, M. R.; GAMBOA R.; JASSO, D.; RODRÍGUEZ, R. 2003a. Fungitoxic effect of *Larrea tridentata* resin extracts from the Chihuahuan and Sonoran mexican deserts on *Alternaria solani*. Agrochimica 47: 50-60.

LIRA-SALDIVAR, R. H.; HERNÁNDEZ-CASTILLO, F. D.; GAMBOA-ALVARADO, R.; JASSO DE RODRÍGUEZ, D.; JIMÉNEZ-DÍAZ, F. 2003b. 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. Revista Mexicana de Fitopatología 21: 97-101.

LORITO, M.; DI PIETRO, A.; HAYES, C. K.; WOO, S. L.; HARMAN, G. E. 1994. Antifungal synergistic interaction between chitinolitic enzymes from *Trichoderma harzianum* and *Enterobacter cloacae*. Phytopathology 83: 721-728.

MUZZARELLI, R. A. A. 1986. Filmogenic properties of chitin/chitosan, pp. 389-396. *In*: MUZZARELLI, R.C.; JEUNIAUX, G.W. (Eds.), Chitin in Nature and Technology. Plenum Press, New York, USA.

RIVERA-CASTAÑEDA, G.; MARTÍNEZ, M. A, VALLEJO, S.; ÁLVAREZ, G.; VARGAS, I. C.; MOYA, P.; YÚFERA, P. 2001. *In vitro* inhibition of mycelial growth of *Tilletia indica* by extracts of native plants from Sonora, México. Revista Mexicana de Fitopatología 19: 214-217.

RHOADES, D.F. 1977. Integrated antiherbivore, antidesiccant and ultraviolet screening properties of creosotebush resin. Biochemistry and Systematics Ecology 5: 281–290.

ROMANAZZI, G.; NIGRO, F.; IPPOLITO, A.; DI VENERE, D.; SALERNO, M. 2002. Effect of pre and postharvest chitosan treatments to control storage grey mould of table grapes. Journal of Food Science 67: 1862-1867.

ROMANAZZI, G.; NIGRO, F.; POLITO, A. 2003. Short hypobaric treatments potentiate the effects of chitosan in reducing storage decay of sweet cherries. Postharvest Biology and Technology 29: 73-80.

TSROR (LAHKIM), L.; ERLICH, O.; HAZANOVSKY, M. 1999. Effect of *Colletotrichum coccodes* on potato yield, tuber quality, and stem colonization during spring and autumn. Plant Disease 83: 1253-1258.

VERÁSTEGUI, M.; SANCHEZ, A.; HEREDIA, N.; GARCÍA, A. 1996. Antimicrobial activity of extracts of three major plants from the Chihuahuan desert. Journal of Ethnopharmacology 52: 175-177.

ZHANG, D.; QUANTICK, P. C. 1998. Antifungal effects of chitosan coating on fresh strawberries and raspberries during storage. Journal of Horticultural Science & Biotechnology 73: 763-767.