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Biological efficiency of polyphenolic extracts from pecan nuts shell (*Carya Illinoensis*), pomegranate husk (*Punica granatum*) and creosote bush leaves (*Larrea tridentata* Cov.) against plant pathogenic fungi

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ABSTRACT

Bioactive compounds extracted from plants or agro-industrial residues have great potential as novel fungicide sources for controlling pathogenic fungi. In this study antifungal activity of polyphenolic extracts from *Larrea tridentata* leaves, *Carya illinoensis* shells and *Punica granatum* husk were evaluated *in vitro* against eight different plant pathogenic fungi and ten isolates of *Fusarium oxysporum*. Phenolic solutions of gallic and ellagic acids were also tested at different concentrations. The polyphenolic extracts tested have a high efficiency to inhibit the mycelial growth of *Pythium sp.*, *Colletotrichum truncatum*, *Colletotrichum coccodes*, *Alternaria alternata*, *Fusarium verticillioides*, *Fusarium solani*, *Fusarium sambucinum*, and *Rhizoctonia solani*. *L. tridentata* polyphenolic extracts also efficiently inhibited the mycelial growth of eight out of ten *F. oxysporum* isolates. These results showed that the polyphenolic extracts tested possess antifungal activities against a broad spectrum of plant pathogenic fungi and could be used as potential antifungal agents for the control of fungal plant diseases.

1. Introduction

Phytopathogenic organisms cause a wide spectrum of diseases in plants and include fungi, nematodes, bacteria, and viruses (Montesinos, 2003). Plant pathogenic fungi cause losses in numerous economically important crops (Fletcher et al., 2006). Hussein et al. (2002) found that most of grains in maize fields were infected with *Fusarium* species, and soil can be considered as one of the most important inoculum sources for these species. *Pythium sp.*, and *Alternaria alternata* are two fungi of worldwide distribution. Sankaran et al. (2005) reported that fungus like *Ganoderma* causes a decrease of production and death of various plants, such as cash crops and trees in India. Several fungi have been found to induce post-harvest spoilage of fruits and vegetables, which is associated with decrease in nutritive elements (Ray and Ravi, 2005). Diseases caused by fungi are also a serious problem in forest management. Damping-off of seedlings caused by *Rhizoctonia solani* were frequently observed on many woody perennial plants (Chang, 1997). *Colletotrichum gloeosporioides* causes anthracnose disease of trees and results in leaf spots and defoliation (Chang et al., 1997). Trees infected with *Fusarium solani* showed root crown rot, dieback and wilt (Fu and Chang, 1999; Demirci and Maden, 2006).

Chemical treatments of infested soils are one of the main solutions for plant pathogenic disease control (Montesinos, 2003; Nunes et al., 2001). Synthetic fungicides are helpful to sustaining crop production by protecting plants from fungal diseases. However this agronomic practice faces new demands by the consumers, particularly for organic and chemically untreated products. In addition, producers and farmers are worried by the resistance of phytopathogenic microorganisms to fungicides due this is one of the critical causes of poor disease control in agriculture (Aguin et al., 2006; Ishii, 2006). There are, therefore, needs to develop alternative agents for the control of pathogenic fungal diseases in plants (Prabavathy et al., 2006; Chang et al., 2008).

Several studies on the fungi-toxic activities of plant secondary metabolites have been reported (Muller-Riebau et al., 1995; Ojala et al., 2000; Kordali et al., 2003; Nunez et al., 2006; Field et al., 2006; Lee, 2007). Tannin-rich plants of the semiarid regions of Mexico have in most cases a pool of unknown and well-defined phytochemicals with antimicrobial potential to be used against plant pathogenic fungi. Lira-Saldivar et al. (2003) reported that the extract of *Larrea tridentata* Cov. was effective against *Pythium* sp.

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Ventura-Sobrevilla et al. (2006) reported that extracts of *Fluorensia cernua*, *Jatropha dioica*, *Turnera diffusa* and *Euphorbia antisyphilitica* were effective against some important fungi including *Penicillium purpurogenum*, *Fusarium spp.*, *A. alternata*, *R. solani* and *Aspergillus flavus*. The chemical composition of *L. tridentata* and *F. cernua* and its tannin composition have been previously reported by our research group (Belmares et al., 2009). Also, we have put special attention on the chemical structure and diversity of the tannins and their biodegradation trying to develop bioprocesses that permit the biotransformation of the tannins present in some plants of semiarid region of Mexico and produce important phenolic bioactivities (Aguilera-Carbo et al., 2009). In this study we report the antifungal activity of polyphenolic extracts from creosote bush leaf (*L. tridentata* Cov.), pecan nuts shell (*Carya Illinoensis*) and pomegranate husk (*Punica granatum*) against plant pathogenic fungi.

2. Methods

2.1. Plant materials

L. tridentata Cov. leaves were collected in the Experimental Station of The Universidad Autonoma Agraria "Antonio Narro", Buenavista, Saltillo, Coahuila, Mexico during August 2007. The ripening pomegranate fruits used in this study were acquired from an orchard located in the region of Sabinas, Coahuila, Mexico during September 2007. Pecan nut shells were collected during October 2007 in a farm located in the region of Parras, Coahuila, Mexico. Shells were collected (endocarp) when fruit was ripening and ready to be used as food. Both nut shells and pomegranate husks are considered a by-pass product. It its known that polyphenols content may varies according plant age, season, storage, harvest time and many other factors more. The three vegetal materials were dehydrated to 60 °C for 48 h. Samples were pulverized to a 30 mesh particle size in an industrial homogenizer (5 l; model LP12 Series 600-182, JR Maquinaria para mercado S.A. de C.V., Mexico).

2.2. Plant pathogenic fungi

Eight plant pathogenic fungi were selected to be used in the present study and obtained from International Centre of Phytosanitary Services (CISIF) Collection (Saltillo Coahuila, Mexico). *Pythium sp.* (1), *Colletotrichum truncatum* (2), *Colletotrichum coccodes* (3), *A. alternata* (4), *Fusarium verticillioides* (5), *F. solani* (6), *Fusarium sambucinum* (7) and *R. solani* (8) which are usual damping-off pathogens of plants, causing many leaf and root diseases were used in this study. Each fungal strain was cultured in potato dextrose agar (PDA, Difco Company) medium. *Fusarium* strains were isolated from samples of infected chili peppers (*Capsicum annum* L.) harvested in Fresnillo, Zacatecas, Mexico. All fungal strains were purified by single-spore cultures on potato dextrose agar medium.

2.3. Extraction of polyphenols

Extraction of polyphenolic fraction from *L. tridentata* was following the method reported previously by Ventura et al. (2008): a mass of 100 g of dried powder was placed in an Erlenmeyer flask with 400 mL of 70% acetone. The flask was covered with aluminum foil to avoid light exposure. This mixture was refluxed at 60 °C for 12 h. After this process, the sample was filtered using Whatman filter paper no. 41 and centrifuged at 3500 rpm for 15 min. The solvent was removed using a rotary evaporator (Yamato, RE540) using a temperature below 60 °C and by avoiding light exposure. Polyphenolic extract of *P. granatum* was obtained as follows: a mass of 150 g dried powder was placed in an Erlenmeyer flask with 750 mL of water. The flask was covered with aluminum foil to avoid light exposure. This mixture was refluxed at 60 °C for 12 h. After this process, the sample was filtered using Whatman filter paper no. 41 and centrifuged at 3500 rpm for 15 min. For pecan nut shells, the polyphenolic fraction was obtained following the same protocol reported for *L. tridentata* leaves changing the solvent by 70% methanol.

2.4. Monomeric phenolics and polyphenolic extracts concentrations

Aqueous solutions of gallic and ellagic acids (Sigma reagent) were prepared at four concentrations (125, 250, 500 and 1000 ppm) and maintained in black bottles at 4 °C until their use in the antifungal activity assay. Polyphenolic solutions of extract of *L. tridentata*, *P. granatum* and *C. illinoensis* were prepared at concentrations of 0.02, 0.05, 0.10 and 0.20 ppm.

2.5. Conditions of antifungal assay

All micro-assays were evaluated in conventional microplates of polystyrene, which were sterilized by autoclaving at 10 pounds per 20 min. Each well of the microplate was filled with 200 μL of sterile Sabouraud dextrose agar and inoculated with 20 μL of spores suspension (at a concentration of 2×10^7 fungal spores per mL). After, 20 μL of polyphenolic extracts or monomeric phenolics solution were added. A control treatment (sterile water) was included in the test. Inoculated microplates were incubated at 30 °C during 24–48 h. Fungal growth was stereoscopically monitored with observations each 6 h, using a stereoscope Zeiss Olympus SZ30.

2.6. Experimental design and data analysis

A nonparametric one way ANOVA design was used to evaluate the effect of each of the factors (the polyphenolic solutions, their concentrations and the fungal strains) on antifungal activity. Five replicates were evaluated in each assay. Experimental unit per replication were 10 plate wells. Fungal growth inhibition was evaluated as qualitative trait (presence and absence). If the fungus grew on all plate wells, it was considered 0% of inhibition and so on. Data were ranked as 1 = 0% growth inhibition, 11 = 100% growth inhibition; each group of data was analyzed individually by PROC NPAR1WAY using the software SAS version 6.03. The significance value that was used to reject the null hypothesis was $p = <0.05$. Fungal species were grouped according their sensibility to plant extracts and monophenols (Wilcoxon test). Extracts and monophenols were arbitrarily grouping according to their fungicide capacity.

3. Results and discussion

In this study, the antifungal activity of polyphenolic and monomeric phenolic extracts were tested against well-known phytopathogenic fungi. Results of biological activity of these extracts against eight fungal strains showed that *L. tridentata* polyphenolic extracts had a strong fungicide effect on the growth of *Phytium sp.*, *C. coccodes*, *C. truncatum*, *A. alternata*, *F. solani* and *R. solani* (Table 1). *F. verticilloides* was only affected by the fungistatic activity of the extracts. Fungicide activity was considered when no fungal growth was observed in the plates and fungistatic activity was considered when fungal growth was delayed. These results are according with those reported by Lira-Saldivar et al. (2003), these authors mentioned the fungicide activity of extracts of *L. tridentata* against *Phytium* species. Our results show clearly the *L. tridentata* extracts exhibit a biological activity against some Oomycetes and Deuteromycetes.

Pecan nut shell polyphenolic extracts inhibited the growth of *Phytium sp.*, *C. coccodes*, *C. truncatum*, *F. sambucinum* and *R. solani*, while a fungistatic effect was evidenced against *A. alternata*, *F. solani*