

Resistance of cactus pear (*Opuntia ficus-indica*) against *Pseudocercospora opuntiae* through β -1,3-glucanase activity and polyphenolic compounds in cladodes

M.J. Ochoa¹, L.M. González-Flores², J.M. Cruz-Rubio², L.A. Rivera-López², S. Rodríguez³, M.A. Nazareno³ and J.F. Gómez-Leyva^{2,a}

¹Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero, Avda. Belgrano (S) 1912 (4200), Santiago del Estero, Argentina; ²TecNM-Instituto Tecnológico de Tlajomulco, Km 10 Carretera a San Miguel Cuyutlán, Tlajomulco de Zúñiga, Jalisco 45640, Mexico; ³CITSE-CONICET-UNSE, RN 9 Km 1125, Santiago del Estero, Argentina.

Abstract

Black spot disease, caused by the hemibiotrophic fungus *Pseudocercospora opuntiae*, is one of the main phytosanitary problems of cactus (*Opuntia* spp.). Through mass selection, one cultivar of *Opuntia ficus-indica* (L.) Mill. resistant to colonization by *P. opuntiae* was identified. The ethanolic extract of resistant cladodes showed higher levels of total condensed tannins, flavonoids and polyphenols than those of the susceptible genotypes, generating 93% inhibition of *P. opuntiae* conidial germination in vitro. The total protein in the resistant genotype showed 300% higher β -1,3-glucanase than the susceptible genotype. This increased activity was able to inhibit germination of conidia by 90%, a similar effect to that of the fungicide Captan® (*N*-trichloromethylthio-4-cyclohexene 1,2-dicarboximide). It was shown, for the first time, that the combined action of cactus polyphenols and β -1,3-glucanase contributes significantly to resistance against *P. opuntiae*. Activity of this enzyme and the phytochemical profile can be used as criteria to predict and detect cactus germplasm with resistance to black spot.

Keywords: pathogenesis-related-proteins, fungal disease, antifungal protein, cactus pear, phenolic compounds

INTRODUCTION

In the 1990s, a new disease was detected in Mexican cactus pear fields. It was named black spot, and its causative agent was found to be *Pseudocercospora opuntiae* (Ayala-Escobar et al., 2006; Quezada-Salinas et al., 2006; Ochoa et al., 2015). The severity and epidemiology of this disease are on the rise, as the cactus pear reproduces asexually, causing a loss in genetic diversity (Méndez-Gallegos et al., 2003).

To date, the most effective forms of control of this disease are the use of resistant genotypes of *Opuntia*, as well as the application of preventive measures (e.g., removing the infected cladodes from the plantation fields). *P. opuntiae*-resistant genotypes have been found by sampling in several commercial plantations in Jalisco state, Mexico; however, the cause of this resistance is yet unknown (Ochoa et al., 2015).

P. opuntiae invades the intracellular spaces and, in leaves, the infection advances through the nervures (Monda et al., 2001). It is known that this pathogen releases a diffusible product (most likely a toxin) that damages the chloroplast and plasmatic membranes, causing both chlorosis and necrosis. This damage allows the fungus access to host nutrients (Monda et al., 2001). The formation of stromata after the destruction of the host cells allows the survival of the fungus, remaining inactive until favorable conditions for sporulation occur.

Recent studies have reported that *P. opuntiae* develops in the epidermis of the collenchyma and chlorenchyma. After 111 days of infection, the hyphae extend to the storage

^aE-mail: jfgleyva@hotmail.com



parenchyma, vascular bundles and medullary parenchyma. After 122 days, plasmolysis in the collenchyma and chlorenchyma occurs, as well as destruction of the chloroplasts in the substomatal cavity and the formation of stromata begins (Quezada-Salinas et al., 2013).

The resistance of plants to disease often results from the interaction of specific resistance genes in the plant with the corresponding avirulence genes in the pathogen (García and Lozoya, 2004). These resistance genes code for different groups of proteins related to defense against pathogens. Among these are the pathogenesis-related (PR) proteins, which degrade the cell wall of the invading pathogen. These enzymes include β -1,3-endoglucanases and chitinases, which are promoted in infectious processes (van Loon et al., 2006). These enzymes are induced by different kinds of pathogens and abiotic stresses (van Loon, 1999).

There is little information available regarding resistance to biotic factors in *Opuntia*. In the *Cactaceae*, Treviño et al. (2012) reported antifungal activity of methanolic extracts of *Stenocereus pruinosus* and *Echenocereus stramineus* on dermatophytic fungi. In *Opuntia*, Sharavna Kumar et al. (2013) reported antibacterial and antifungal activity of methanolic extracts of *Opuntia dillenii* against *Aspergillus niger*, *Candida albicans*, *Monilia fruticola*, *Auricularia polytricha*, *Chaetomella raphigera* and *Athrobotrys olidospora*. Bergaoui et al. (2007) found that the chemical composition of extracts of flowers, fruits and especially the cladodes of *Opuntia microrhiza* have an effect against *Alternaria solani*.

Recently, the activities of chitinase and β -1,3-glucanase and lignin content in mango (*Mangifera indica* L.) leaves were related to the resistance to flower malformation. Ebrahim et al. (2011) revealed that the chitinase and β -1,3-glucanase activity in leaves correlated with disease resistance. Therefore, chitinase and β -1,3-glucanase activities are very likely to contribute to disease resistance and, as such, their presence can be used as criteria to predict and detect resistant germplasm.

β -1,3-Glucanases have also been related to resistance to *Pseudocercospora*. *Arachis hypogaea* plants were transformed with a gene for β -1,3-glucanase, coding for a 38-amino-acid protein (38.8 kDa), and showed resistance to *Cercospora personata* (Sundaresha et al., 2010; Qiao et al., 2014). In *Beta vulgaris* inoculated with chitosan in order to induce defense mechanisms, β -1,3-glucanase accumulated and, with it, the damage caused by *Cercospora beticola* diminished (Felipini and Di Piero, 2013).

It is well known that plants synthesize a variety of secondary metabolites, biologically active compounds, in their tissues. Some have antifungal properties that stop or inhibit mycelial growth and/or inhibit germination, or reduce the sporulation of pathogenic fungi. Each metabolite group has different action mechanisms: for example, the toxicity of polyphenols to microorganisms is attributed to enzymatic inhibition by oxidation mechanisms. The main function of polyphenols is related to the defense of plants against pathogen attack. The resistance can come from the bactericide/bacteriostatic/antifungal properties of the polyphenol itself, or from the generation of oxidation products of these (quinones and melamines) that have marked antimicrobial characteristics (Osorio et al., 2010).

Pectins, carotenes, betalains, ascorbic acid and quercetin and its derivatives have been found in the fruit of *Opuntia ficus-indica*. The antioxidant effects and the benefits to human health of these compounds have been extensively studied (Coria Cayupán et al., 2011). However, they have not been related to benefits to the plant itself or against its pathogens. Quezada-Salinas et al. (2013) observed the presence of polyphenols in response to invasion by *Pseudocercospora*. With this background, the aim of this study was to study antifungal properties in the cladodes of resistant (R) and susceptible (S) genotypes of *O. ficus-indica* to *P. opuntiae* by the determination of β -1,3-glucanase and chitinase activities and total content of phenolic compounds, flavonoids and tannins.

MATERIALS AND METHODS

Biological material

P. opuntiae was obtained from commercial plantations in Jalisco state, Mexico. The

inoculum used was prepared from colonies grown in 2% malt extract medium (MEA) after 60 days of growth. The conidial solution was standardized to 1×10^6 conidia mL⁻¹.

Ethanolic extract preparation

Cladodes of *O. ficus-indica* were chosen by mass selection and based on previous pathogenicity studies (twice inoculated with the pathogen). They were categorized as resistant (R) or susceptible (S) according to the method of Daub (1986). The cladodes were superficially disinfected and dried at $40 \pm 2^\circ\text{C}$. Once dried, they were ground to a fine powder. Samples weighing 10 g dry matter were mixed with 80% (v/v) ethanol at a 1:10 ratio (w/v) and kept under constant agitation for 48 h. The mixture was filtered and the filtrate was concentrated by evaporation of the ethanol in a rotary evaporator at 40°C and reduced pressure. The aqueous concentrate was filtered (0.25 mm) and lyophilized.

Phenolic and flavonoid content

Two hundred milligrams lyophilized extract was extracted with 10 mL methanol using a tissue homogenizer. The extraction vessel was placed in an ultrasonic bath for 10 min, followed by centrifugation at 10,000 rpm for 10 min, and the supernatant was retained. The precipitate was extracted once more under the same conditions, and both supernatants were pooled and standardized to 20 mL total with methanol.

The total phenolic content of the extract was determined by the Folin-Ciocalteu method. Gallic acid was used as the reference compound. The total amount of phenolic compounds is expressed as equivalents of gallic acid in mg g⁻¹ dry matter. The total flavonoid content of extracts was determined by the aluminum chloride colorimetric method (Chaillou and Nazareno, 2009).

Total condensed tannin content

Condensed tannins are degraded in acidified butanol, producing colored anthocyanin-like compounds. An aliquot of 10 mL butanol/HCl (95:5) was added to 200 mg dry material in a test tube and the mixture was homogenized in a tissue homogenizer. The solution was heated for 60 min at 95°C and left to cool to room temperature. This was transferred to a 10-mL volumetric flask and the volume was adjusted with butanol. The absorbance was read at 550 nm, and the reference compound used was cyanidin 3-glucoside ($\epsilon = 26900 \text{ M}^{-1} \text{ cm}^{-1}$) (Salcedo et al., 2010).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

A solution containing DPPH radicals loses color in the presence of antioxidant compounds. DPPH was dissolved in methanol until an absorbance of 1.00 was obtained. An aliquot of 3 mL of this solution was placed in the cuvette (1 cm path length) and the loss of absorbance was monitored at 515 nm for 20 cycles for 10 min. The stationary value of the absorbance was calculated by adjustment of the exponential decay, as was calculated as percentage of antiradical activity (%AAR). The %AAR was determined for at least five aliquots of each extract in order to express the inhibition concentration as IC₅₀ (González et al., 2010).

Activity of β -1,3-glucanase and chitinase

Activity of β -1,3-glucanase (EC 3.2.1.14) was determined according to Ozgonen et al. (2009). The amount of glucose liberated from laminarina for the enzyme present in the cladode extract was determined spectrophotometrically at 515 nm using 3,5-dinitrosalicylic acid (DNS). The activity of β -1,3-glucanase was expressed in U mg⁻¹ protein g⁻¹ dry weight. An enzyme unit was defined as the amount of enzyme that catalyzes the release of sugar-reducing groups equivalent to 1 mmol glucose min⁻¹. Measurements were taken in five repetitions. Chitinase activity in the extract of the cladodes was analyzed in accordance with the procedure laid down by Molano et al. (1977) using 4-dimethylamino benzaldehyde (4-DMABA), determined spectrophotometrically at 585 nm. Chitinase activity was expressed in nanokatal g⁻¹ fresh weight. The measurements were taken in five repetitions. A colorimetric



method was used to measure the activity of chitinases. Enzyme activity was determined for each of the protein fractions of the total extract of R and S genotypes of *Opuntia*, precipitated with ammonium sulfate at 0-100, 0-40, and 40-70% saturation (SAT).

Evaluation of fungicidal activity against *P. opuntiae*

A 96-well ELISA plate was used, with extract at concentrations of 5, 10, 15, 20 mg mL⁻¹ of R and S extract, and as antifungal control Captan® (*N*-trichloromethylthio-4-cyclohexene 1,2-dicarboximide) at 0.2 g mL⁻¹. The treatments were added to 50 µL *P. opuntiae* inoculum at a concentration 1×10⁻⁶ conidia mL⁻¹, reaching a final volume of 350 µL. The trial was conducted under 26°C and 16/8 h light/dark conditions for 30 days.

Effect of protein extracts on germination inhibition of *P. opuntiae*

In a second experiment, the in-vitro effectiveness of protein fractions of *Opuntia*, precipitated with ammonium sulfate at 0-100, 0-40, or 40-70% SAT, was tested on conidia and hyphae germination of *P. opuntiae*. For this experiment, 0.2-mL tubes were used. In each tube, protein extract from different fractions was added at 25 and 50 mg mL⁻¹, with 25 µL conidial suspension of the pathogen at 11.6×10⁻⁶ conidia mL⁻¹. Captan® (0.2 g mL⁻¹) was used as a positive control, and 2% MEA was used as a negative control. The tubes were placed at 26°C for 72 h. After this incubation period, germinated conidia in each of the treatments were counted in a Neubauer chamber. Images were obtained of the morphology of hyphae and conidia in each of the treatments.

Statistical analysis

At least three replicates per determination were carried out in all experiments. Data were analyzed statistically with *t*-test and analysis of variance (ANOVA). Tukey's test was used for all multiple comparisons at p≤0.05.

RESULTS AND DISCUSSION

Phytochemical profile of *O. ficus-indica* cladodes

There were significant differences between the R and S genotypes for all analyzed metabolites (condensed tannins, total phenolic compounds, flavonoids, antiradical activity against DPPH) (Table 1). Condensed tannins, flavonoids and total phenolic compounds concentration (mg g⁻¹) were higher in the R genotype.

Table 1. Concentration of phenolic compounds and antioxidant activity in ethanol extracts of *O. ficus-indica* genotypes and antifungal activity against *P. opuntiae*. Values are means ± standard deviations.

Property	Resistant	Susceptible
Condensed tannin (mg eq. cyanidin 3-glucoside g ⁻¹ DW)	1.56±0.23	0.48±0.09
Total phenols (mg eq. gallic acid g ⁻¹ DW)	168±9	23.1±3
Flavonoids (mg eq. quercetin g ⁻¹ DW)	55±9	15±4
IC ₅₀ of antiradical activity (DPPH mg mL ⁻¹)	0.52±0.02	1.04±0.04
Inhibition of germination (%)	93.34±1.2	14.20±2.6

Tannins were 3.5 times higher in R genotypes, while total phenols and flavonoids were 7 and 3.5 times more, respectively; the antioxidant activity increased by 50% in the R extract. The inhibitory activity on conidial germination of *P. opuntiae* in the R genotype was 93% compared with 14% in the S genotype; this inhibition value similar to that of the antifungal compound Captan® (Figure 1).

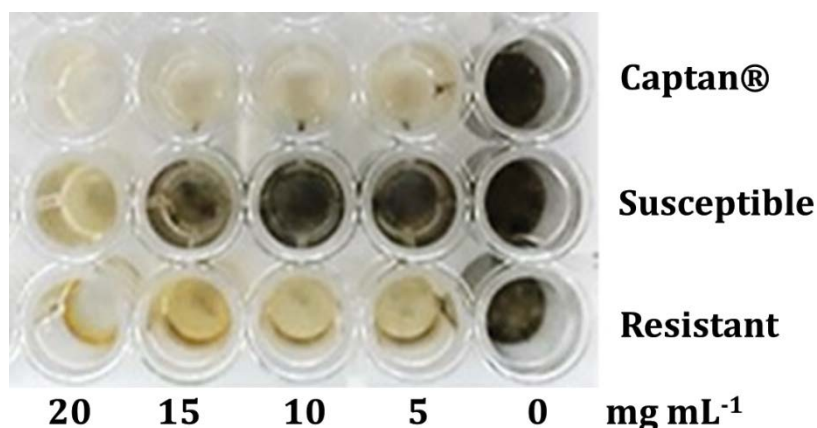


Figure 1. Evaluation of antifungal activity of *Opuntia ficus-indica* ethanolic extract against conidial germination of *Pseudocercospora opuntiae*.

Sharavana Kumar et al. (2013) reported antifungal activity associated with flavonoids from ethanol extracts of *O. dillenii*. They reported that the accumulated phenols pass to the cell wall by the action of peroxidases that were part of the lignin, cutin and suberin and act as mechanical support and barriers against microbial invasion. They can also accumulate as inducible low-molecular-weight compounds called phytoalexins, as a response to microbial attack. It is stressed that these compounds are of a post-infection nature; although they may be present previously at low concentrations in the plant, they accumulate rapidly as induced compounds after an injury or a pathogen attack.

In the case of black spot, Quezada-Salinas et al. (2013) observed an increase of polyphenols in *Opuntia* as response to invading mycelium of *Pseudocercospora*, 129 days after inoculation, causing black-colored tissues. These results are related to a resistance response to the necrotrophic stage of the pathogen.

Two phenolic compounds, piscidic and eucomic acid, in ethanol extracts of *O. ficus-indica* and 16 flavonoids, including glycosides of quercetin, kaempferol and isorhamnetin (Ginestra et al., 2009), were identified. These same flavonoids present in *Asparagus officinalis* extracts showed antifungal activity against *Fusarium oxysporum* (Rosado-Álvarez et al., 2014). Based on the above, it can be considered that the resistance of the R genotype may be related to increased production of preformed phenolics and flavonoids; i.e., production could be higher in developing tissues of this genotype.

Enzymatic activity of β -1,3 glucanases and chitinases

No activity of chitinase was identified in either genotype (data not shown). Higher glucanase activity was registered in cladodes of the R genotype compared with S, 10.3 and 4.95, respectively (Table 2). Previous studies have shown that the production of glucanases has been linked to the selection of resistant plants to *Pseudocercospora* and *Cercospora* (Sundaresha et al., 2010; Felipini and Di Piero, 2013; Qiao et al., 2014).

Protein extracts on inhibiting germination of *P. opuntiae*

The in vitro study of the effectiveness of protein fractions of *Opuntia* on the inhibition of germination of conidia of *P. opuntiae*, showed greater inhibition values of R genotype protein fractions, compared with those of genotype S (Table 2). The inhibition level of R genotype was close to 90%, value surpassed only by inhibition generated by fungicide Captan®. Evidence suggests that plant extracts can be used against microorganisms that cause diseases in plants. High percentages of *Cercospora circinans* conidia inhibition were reached after applying extracts of resistant potato cultivars, rich in phenolic compounds. The higher production of phenols and glucanases of genotype R may be related to its inhibition capacity of conidial germination. The effects on conidial germination inhibition of genotype R, is related to structural damage of conidial morphology (Figure 2). Conidia that were in

contact with the R genotype extract showed detachment of the cell wall, accumulation of lipid compounds and dehydration; none of the conidia exposed under these conditions shows the formation of the germ tube. These damages are also observed in conidia in contact with Captan®.

Table 2. Glucanase activity and inhibition of germination of *Pseudocercospora opuntiae* conidia in protein fractions of *Opuntia ficus-indica* genotypes. Values are means \pm standard deviations.

Genotype	NH ₄ (SO ₄) ₂ saturation (%)	β -1,3-Glucanase (U mg ⁻¹ protein)	Inhibition of germination (%)
Resistant	0-40	8.53 \pm 0.09 bc	72.9 \pm 0.13 b
	40-70	12.80 \pm 0.95 a	89.6 \pm 0.69 a
	0-100	9.58 \pm 0.56 b	84.0 \pm 0.49 ab
Susceptible	0-40	3.99 \pm 0.23 e	23.3 \pm 0.67 de
	40-70	5.90 \pm 0.28 d	28.5 \pm 0.12 d
	0-100	3.90 \pm 0.49 e	38.2 \pm 0.83 c

Values followed by different letters within columns are statistically different by Tukey's test at $p \leq 0.05$.

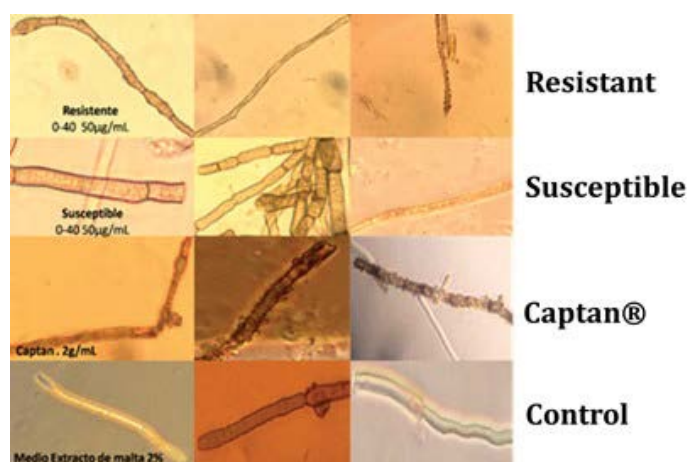


Figure 2. Effect of protein extracts of *Opuntia ficus-indica* cladodes on germination and conidia morphology of *Pseudocercospora opuntiae*.

CONCLUSIONS

This work reports the biochemical and phytochemical profiles of R and S genotypes of cactus pear (*O. ficus-indica*) against *P. opuntiae*. Production of condensed tannins, flavonoids, and total phenolic compounds in cladodes was greater in the R genotype compared with the S genotype. Our results showed that the R genotype showed nearly 50% higher glucanase activity compared with the S genotype. Antifungal activity present in ethanolic extracts of cladodes showed that the R genotype causes 93.24% growth inhibition of conidia germination after 72 h in vitro. This activity was related to structural damage in conidial morphology and inhibition of germination. Based on these results, it is suggested that the R genotype presents constitutively early defense mechanisms in fungal diseases. The information generated can be useful as a phytochemical and biochemical resistance marker in the selection of cactus pear genotypes against *P. opuntiae*.

Literature cited

- Ayala-Escobar, V., Yañez-Morales, M.J., Braun, U., Groenewald, J.Z., and Crous, P.W. (2006). *Pseudocercospora opuntiae* sp. nov., the causal organism of cactus leaf spot in Mexico. *Fungal Divers.* 21, 1–9.
- Bergaoui, A., Boughalleb, N., Ben Jannet, H., Harzallah-Shiric, F., El Mahjoub, M., and Mighri, Z. (2007). Chemical

- composition and antifungal activity of volatiles from three *Opuntia* species growing in Tunisia. *Pak. J. Biol. Sci.* **10** (15), 2485–2489 <https://doi.org/10.3923/pjbs.2007.2485.2489>. PubMed
- Chaillou, L.L., and Nazareno, M.A. (2009). Bioactivity of propolis from Santiago del Estero, Argentina, related to their chemical composition. *Lebensm. Wiss. Technol.* **42** (8), 1422–1427 <https://doi.org/10.1016/j.lwt.2009.03.002>.
- Coria Cayupán, Y.S., Ochoa, M.J., and Nazareno, M.A. (2011). Health-promoting substances and antioxidant properties of *Opuntia* sp. fruits. Changes in bioactive compound contents during ripening process. *Food Chem.* **126** (2), 514–519 <https://doi.org/10.1016/j.foodchem.2010.11.033>.
- Daub, M.E. (1986). Tissue culture and the selection of resistance to pathogens. *Annu. Rev. Phytopathol.* **24** (1), 159–186 <https://doi.org/10.1146/annurev.py.24.090186.001111>.
- Ebrahim, S., Usha, K., and Singh, B. (2011). Pathogenesis related (PR) proteins in plant defense mechanism. In *Science Against Microbial Pathogens: Communicating Current Research and Technological Advances*, A. Méndez-Vilas, ed. (Badajoz, Spain: Formatex Research Center), p.1043–1054.
- Felipini, R.B., and Di Piero, R.M. (2013). PR-protein activities in table beet against *Cercospora beticola* after spraying chitosan or acibenzolar-*S*-methyl. *Trop. Plant Pathol.* **38** (6), 534–538 <https://doi.org/10.1590/S1982-56762013000600009>.
- García, E., and Lozoya, E.G. (2004). Genes de resistencia a enfermedades en plantas. *Rev. Mex. Fitopatol.* **3**, 414–422.
- Ginestra, G., Parker, M.L., Bennett, R.N., Robertson, J., Mandalari, G., Narbad, A., Lo Curto, R.B., Bisignano, G., Faulds, C.B., and Waldron, K.W. (2009). Anatomical, chemical, and biochemical characterization of cladodes from prickly pear [*Opuntia ficus-indica* (L.) Mill.]. *J. Agric. Food Chem.* **57** (21), 10323–10330 <https://doi.org/10.1021/jf9022096>. PubMed
- González, E.A., García, E.M., and Nazareno, M.A. (2010). Free radical scavenging capacity and antioxidant activity of cochineal (*Dactylopius coccus* C.) extracts. *Food Chem.* **119** (1), 358–362 <https://doi.org/10.1016/j.foodchem.2009.06.030>.
- Méndez-Gallegos, S., Panzavolta, J.T., and Tiberi, R. (2003). Carmine cochineal *Dactylopius coccus* Costa (*Rhynchota: Dactylopiidae*): Significance, production and use. *Adv. Hort. Sci.* **17** (3), 165–171.
- Molano, J., Durán, A., and Cabib, E. (1977). A rapid and sensitive assay for chitinase using tritiated chitin. *Anal. Biochem.* **83** (2), 648–656 [https://doi.org/10.1016/0003-2697\(77\)90069-0](https://doi.org/10.1016/0003-2697(77)90069-0). PubMed
- Monda, E.O., Sanders, F.E., and Hick, A. (2001). Infection and colonization of bean leaf by *Phaeoisariopsis griseola*. *Plant Pathol.* **50** (1), 103–110 <https://doi.org/10.1046/j.1365-3059.2001.00537.x>.
- Ochoa, M.J., Rivera, L.A., Arteaga-Garibay, R.I., Martínez Peña, M.D., Ireta, J., and Portillo, L. (2015). Black spot caused by *Pseudocercospora opuntiae* in cactus pear productive systems of Jalisco, Mexico. *J. Prof. Assoc. Cactus Dev.* **17**, 1–12.
- Osorio, E., Flores, M., Hernández, D., Ventura, J., Rodríguez, R., and Aguilar, C. (2010). 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. *Ind. Crops Prod.* **31** (1), 153–157 <https://doi.org/10.1016/j.indcrop.2009.09.017>.
- Ozgonen, H., Yardimci, N., and Kilic, H.C. (2009). Induction of phenolic compounds and pathogenesis-related proteins by mycorrhizal fungal inoculations against *Phytophthora capsici* Leonian in pepper. *Pak. J. Biol. Sci.* **12** (17), 1181–1187 <https://doi.org/10.3923/pjbs.2009.1181.1187>. PubMed
- Qiao, L.X., Ding, X., Wang, H.C., Sui, J.M., and Wang, J.S. (2014). Characterization of the β -1,3-glucanase gene in peanut (*Arachis hypogaea* L.) by cloning and genetic transformation. *Genet. Mol. Res.* **13** (1), 1893–1904 <https://doi.org/10.4238/2014.March.17.17>. PubMed
- Quezada-Salinas, J., Sandoval, I.S., Alvarado, R.D., and Cardenas, S.E. (2006). Etiología de la mancha negra del nopal (*Opuntia ficus-indica* Mill.) en Tlanepantla, Morelos, México. *Agrociencia* **40**, 641–653.
- Quezada-Salinas, A., Sandoval Islas, J.S., Alvarado Rosales, D., and Moreno Velázquez, M. (2013). Histopatología y patogénesis de *Pseudocercospora opuntiae* en nopal. *Rev. Mex. Micol.* **38**, 9–18.
- Rosado-Álvarez, C., Molinero-Ruiz, L., Rodríguez-Arcos, R., and Basallote-Ureba, M.J. (2014). Antifungal activity of asparagus extracts against phytopathogenic *Fusarium oxysporum*. *Sci. Hort. (Amsterdam)* **171**, 51–57 <https://doi.org/10.1016/j.scienta.2014.03.037>.
- Salcedo, C.L., López de Mishima, B.A., and Nazareno, M.A. (2010). Walnuts and almonds as model systems of foods constituted by oxidisable, prooxidant and antioxidant factors. *Food Res. Int.* **43** (4), 1187–1197 <https://doi.org/10.1016/j.foodres.2010.02.016>.

Sharavana Kumaar, A.P., Vanitha, J., Venkateshwaran, K., Srikanth Reddy, K., and Karthikeyan, D. (2013). Antibacterial and antifungal activity of *Opuntia dillenii* (Cactaceae) fruit extract. *J. Environ. Nanotechnol.* 2 (1), 16–19 <https://doi.org/10.13074/jent.2013.02.121021>.

Sundaresha, S., Manoj Kumar, A., Rohini, S., Math, S.A., Keshamma, E., Chandrashekar, S.C., and Udayakumar, M. (2010). Enhanced protection against two major fungal pathogens of groundnut, *Cercospora arachidicola* and *Aspergillus flavus* in transgenic groundnut over-expressing a tobacco β 1–3 glucanase. *Eur. J. Plant Pathol.* 126 (4), 497–508 <https://doi.org/10.1007/s10658-009-9556-6>.

Treviño, N.J.F., Rodriguez, R.G., Verde, M.J., Morales, M.E., Garza, R.A., and Rivas, C. (2012). Actividad antifungica de *Stenocereus pruinosus* y *Echinocereus stramineus*. *Rev. Mex. Cienc. Farm.* 43 (1), 42–48.

van Loon, L.C. (1999). Occurrence and properties of plant pathogenesis-related proteins. In *Pathogenesis-Related Proteins in Plants*, S.K. Datta, and S. Muthukrishnan, eds. (Boca Raton, FL, USA: CRC Press), p.1–19.

van Loon, L.C., Rep, M., and Pieterse, C.M.J. (2006). Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* 44 (1), 135–162 <https://doi.org/10.1146/annurev.phyto.44.070505.143425>. PubMed