

Allelochemicals from native plants of Argentina: Control of stored grains fungi

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ABSTRACT

Grain rot is caused by *Fusarium* and *Aspergillus* fungal species, these also cause major diseases in cereal crops. They reduce crops yields and contaminate the grains with mycotoxins (secondary metabolites produced by fungi), harmful to health of humans and livestock. Currently these are controlled by fungicides (i.e. azoles) before harvest and by food preservatives (i.e. short chain fatty acids and their salts). These compounds have several disadvantages, hence, are

currently under strong scrutiny. Plant allelochemicals (phenolics) and essential oils are an alternative to replace the present commercial fungicides. These are only a small fraction of all allelochemicals available. This review, describes the current status to control grain rot fungi with plant allelochemicals, to search these compounds and our own research results to use the allelochemicals of native Argentinian plants against *Fusarium* and *Aspergillus* species.

Key words: Aspergillus, essential oils, fungicides, Fusarium, maize, mycotoxins, fungicides, phenolics, wheat,

1. INTRODUCTION

Fungi are a highly diverse group of living organisms, with 144,000 species (18,32). The Ascomycetes and Basidiomycetes have vast fungal diversity (13). Most fungal species are directly or indirectly beneficial to humans (2,32): (i) Several fungal species convert dead animals and plants into nutrients readily available for living plants. (ii) Mycorrhizal fungi establish symbiotic relationships with the root cells to supply nutrients to crop plants. (iii) Several species provide antibiotics (i.e., griseofulvin produced by *Penicillium griseofulvum*) and enzymes required in pharmaceutical industry. (iv) Some species are used in food processing (i.e., *Penicillium roqueforti* required for maturation of cheese) and (v). pest biocontrol (i.e., *Gliocladium* species are used to control plant pathogens). There are only 8000 species of pathogenic fungi, causing 80 % fungal diseases in crops (23) including the molds causing grain spoilage in storage. Fungi not only reduce the grain yields and grains nutritional value but also contaminate with fungal secondary metabolites called mycotoxins, produced from molds growth. These contaminated foods cause severe intoxications in humans and livestock (15). They are invisible, flavourless and survive in storage conditions and food manufacture. Molds are produced by fungi of genus *Aspergillus*, *Fusarium* and *Penicillium* (51). They also infect cereal crops in fields. The *Fusarium* species, synthesize mycotoxins mainly before grain harvest, while, *Aspergillus* and *Penicillium* species are phytopathogens in stored grains (15). This Review briefly provides a background of stored grain rot diseases caused by *Fusarium* and *Aspergillus* species, the problems in their control, use of plants allelochemicals as antifungals and the progress made to identify and characterize these compounds in native plants of Northwest Argentina.

2. GRAIN ROT FUNGI IN ARGENTINEAN MAIZE AND WHEAT

2.1. Effects of Mold species and their mycotoxins on human and animal health

The main toxigenic species reported in Argentina are *Fusarium verticillioides* and *Aspergillus flavus* on maize and *F. graminearum* on wheat and maize (11,27) and the most common mycotoxins are 'Fumonisin'. The *F. verticillioides* produces the most common mycotoxins (62) and Fumonisin B1 is most common fumonisins (Figure 1). They are polyketide derivatives which cause liver and kidney dysfunctions (39). Ingestion of foodstuffs heavily contaminated with fumonisins causes esophageal cancer (14) and leukaencephalomalacia in equines and pulmonary edema in pigs (14). B-trichothecenes produced by *F. graminearum* are also common contaminants of maize grains, especially

deoxynivalenol (DON). The DON intake inhibits the protein synthesis and induces strong oxidative stress (39) and causes diarrhea, emesis, anorexia, feed refusal and growth retardation in farm animals (11) and diarrhea, headaches, abdominal pain, dizziness and fever in humans (25). Other mycotoxins in maize grains are the aflatoxins produced by *A. flavus*, which causes hepatotoxic, teratogenic, mutagenic and carcinogenic effects at very low concentrations. Aflatoxin B1 (Figure 1) is the most potent carcinogenic agent (21) and implicated in hepatic necrosis in equines and primary hepatocellular carcinoma in humans. Most countries including, USA and Europe have set maximum contents for fumonisins, DON and aflatoxins in food and in animal feed (3).

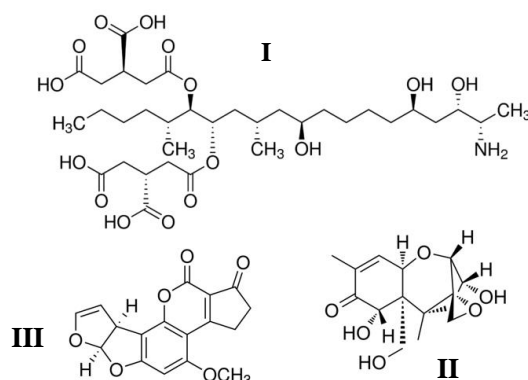


Figure 1. Mycotoxins commonly found in cereal grains: (I) fumonisin B1, (II) deoxynivalenol and (III) aflatoxin B1.

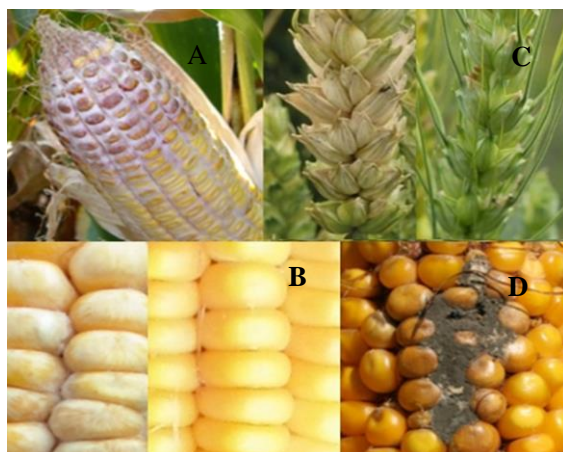


Figure 2. Visible damage caused by *Fusarium* and *Aspergillus* in cereals: (A) Pink cob produced by *Fusarium graminearum* on maize, (B) Star burst symptom in maize caused by *Fusarium verticillioides* (left) compared with healthy grains (right), (C) wheat ear bleaching caused by *F. graminearum* (left) compared with healthy normal ear (right), (D) Spores of *Aspergillus flavus* observed as green powder covering maize grains.

2.2. Visual identification of *Fusarium* and *Aspergillus* grain rots

Grain rot diseases caused by *F. graminearum*, *F. verticillioides* and *A. flavus* can be easily differentiated in the field. *Fusarium graminearum* causes Gibberella ear rot in maize (Figure 2B) and Fusarium head blight in wheat (Figure 2C) while, *F. verticillioides* causes Fusarium ear rot in maize (Figure 2A) (64). *A. flavus* generate the Aspergillus ear rot (22,52).

3. CHEMICAL CONTROL OF GRAIN ROT FUNGI

Currently fungicides (i.e. azoles) and food preservatives (i.e. short chain fatty acids and their salts) are used to prevent mycotoxins accumulation. These compounds have several disadvantages viz., Development of fungal resistance (6) and trigger the accumulation of fumonisins, trichothecenes and aflatoxins (4). Hence, new compounds are needed to overcome these drawbacks of current anti-fungals.

4. PLANT ALLELOCHEMICALS ANTI-FUNGAL ACTIVITIES

Terrestrial plants interact with other living organisms through allelochemicals release (12). Each plant species produces diverse range of allelochemicals for its survival in their habitats. There are 381,910 plant species, only 15 % have been investigated for their secondary metabolites (8). There is likelihood to discover new antifungal allelochemicals in wild vascular plants, hence, the screening for antifungal activity of these plants is urgently needed. Plants produce two types of anti-fungal allelochemicals : Phytoanticipins and Phytoalexins (53).

(i). Phytoanticipins: The highest phytoanticipins concentrations occur in young organs and decrease with maturity (29). Phytoanticipins include several chemical families of natural products [phenolic derivatives, glucosinolates, cyanogenic glycosides, saponins, alkaloids, and non-protein aminoacids (i.e. canavanin)] (53). Most of them are inactive themselves and release antifungal products after enzymatic hydrolysis by specific plant enzymes. Examples are: (i). cyanogenic glycosides in Poaceae (e.g. dhurrin in *Sorghum* species) stem and seed fruit trees, and glucosinolates in Brassicaceae species viz., radish and mustard (e.g. glucobrassicin) (29). However, some phytoanticipins are themselves antifungals. Examples are: the 5-*n*-alkylresorcinols (e.g. 5-*n*-pentadecylresorcinol) present in seed coat of cereal grains (64) and saponins stored in vacuoles (44). Phytoanticipins can be synthesized or accumulated in specific cell types e.g. lipid benzoquinone sorgoleone is produced in the root hairs of *Sorghum* species and the dimeric sesquiterpene gossypol occurs in stem glands in cotton (20,44). The ferulic acid and other phenylpropanoids in pericarp cell walls of cereal grains provides cereal resistance against *Fusarium* species (64). Phytoanticipins are also found in foliar waxes from various cereal species (63).

(ii). Phytoalexins: They are low molecular weight metabolites synthesized after the attack of fungal pathogens (53). Their synthesis is located in the plant cells surrounding the infection site of fungal pathogen. They are quickly accumulated in response to

signals called 'elicitors' from pathogen (i.e. parts of fungal cell walls and fungal proteins) or plant cell wall. The phytoanticipins, phytoalexins are found in some plant families (Fabaceae, Solanaceae, Compositae, Umbelliferae). Fabaceae plants produce isoflavonoid phytoalexins (i.e. daidzein and genistein in bean and in soybean), sesquiterpenes in Solanaceae plant species polycetylenes (i.e. rishitin in potato and tomato) and produce polyacetylenes in Compositae (i.e. thiophenes in sunflower) and Umbelliferae plants (i.e. faltarindiol in carrot) (29,72).

5. PLANT ALLELOCHEMICALS AS ANTIFUNGALS

5.1. Selection of plant species and extraction of antifungal principles

Plants should be selected according to their background in ethnobotany, chemotaxonomy and pharmacology to accelerate the isolation of bioactive molecules and sometimes to prevent the rediscovery of known natural compounds (46). The antifungal principles can be extracted from the selected plants based on traditional extraction methods such as infusion, decoction or tincture. However, extracts recovered by these methods have very complex composition, thus further separation of their antifungal constituent is very difficult. Hence the plant material should be extracted with solvents of increasing polarities (5).

5.2. Screening tests for antifungal activity of plant extracts

The screening tests used to detect the antifungal allelochemicals from plants are based on those developed by the Subcommittee on Antifungal Susceptibility Tests of the Clinical and Laboratory Standards Institute (CLSI) of USA (69). These provide valuable guidelines regarding the selection of microbial strains, composition of culture media, preparation and inocula density, environmental conditions, duration, reading methods and how to define inhibitory response levels. The screening and CLSI tests have strong differences in their design arising from their different purposes. CLSI tests evaluate how sensitive is the population of a human pathogen to an antimicrobial agent, whereas, the screening tests are needed to detect allelochemicals in the plant extracts (present in low concentrations). Some additional guidelines should be considered when designing the screening tests:

(i) Controls: They must include both negative and positive controls (27,69). Negative controls are prepared without the addition of extracts, their molecules or commercial fungicides and are usually available because they allow to quantify the inhibitory effect of the extract or its constituents. Positive controls are commercial fungicides and/or natural products with known antifungal activity, used for comparison of the relative antifungal potency of the extracts or their constituents.

(ii) Organic Solvents: Tests based in the use of liquid hydrophylic media might provide false negatives of antifungal activity for apolar allelochemicals. This is often observed when testing essential oils (41). To overcome this problem, many authors recommend the addition to the culture medium of organic solvents [such as acetone, ethanol, ethylene glycol, methanol, dimethyl sulfoxide and dimethylformamide (19)]

or surfactants such as Tween 20 or 80 (60). However, organic solvents often do not improve solubility in the aqueous media. Addition of surfactant agents to liquid culture can produce foam making pipetting difficult and also generating short-life suspensions or emulsions. Addition of 0.125 % agar to hydrophilic medium increases the medium viscosity and the best solution is needed to provide stable suspensions or emulsions (43).

5.3. Screening tests for antifungal allelochemicals

The main screening tests used are (i) micro-dilution tests, (ii) macro-dilution tests, (iii) Disc diffusion tests, (iv) bioautographic tests based on dot blot and TLC bioautography on silica gel. These will be not described here, as they are already extensively reviewed (4,5,24,27,37,49,69).

6. CHARACTERIZATION OF ANTIFUNGAL ACTIVITY

6.1. *In-vitro* tests

Based on the disease cycles of toxigenic fungi, allelochemicals should be tested on fungal sporulation, spore germination, early hyphae growth and mycelial colonization (5,51). Most of these fungal stages can be evaluated in *in-vitro* tests previously mentioned. However additional tests are needed *in-vitro*, to know the allelochemicals impact on mycotoxin biosynthesis and to explore their modes of action (4,5,27). Testing the combined effects of extracts or extracts in mixture with commercial antifungals can detect synergic interactions and provide important data on differences in the modes of action of natural products with respect to xenobiotics (4, 27).

6.2. *In-vivo* tests

Assays in stored grains are useful, whether the allelochemicals are safe for human health. The antifungals can be added to the grains before or after fungal inoculation, under several combinations of water activity and temperature (49). When allelochemicals are tested as seed protectants, their impact should be evaluated also on seeds germination and seedling growth including ISTA criteria and parameters useful to understand the physiological performance of the young plants (37). The scale of *in-vivo* assays often is restricted due to the low amount of allelochemicals available.

7. SECONDARY METABOLITES TO CONTROL GRAIN ROT FUNGI

7.1. Essential oil

Plant essential oils are complex mixtures of volatile compounds, mainly monoterpenes and sesquiterpenes, that are extracted from the organs (flowers, whole aerial parts, fruits, barks, woods and roots) of aromatic plants (47). These have been extensively reviewed as preservatives of stored grains, to control of *Fusarium* and *Aspergillus* species (47,48). Hence, only main advantages and difficulties are given below:

- (i). The volatile nature of essential oils make it possible to use them as fumigants in cereal grains in closed stores. They have very short residuality, easy biodegradation and less harmful effects on beneficial organisms (65).
- (ii). Fungal resistance is hard to achieve because essential oils at the same time target several physiological processes in fungal cells. Membranes are often primary action sites of essential oils effects (65).
- (iii). Some essential oils under certain *in-vitro* conditions reduce the mycotoxins levels, although the mechanism underlying this effect are not known. Oil constituents inhibits the biosynthetic mycotoxins pathways as in the case of citral and eugenol, which significantly inhibits the ochratoxin A produced by *Aspergillus ochraceus* (33). Another explanation could be that some essential oils degradate mycotoxins. Examples are palmarosa and lemon oils, which reduces the DON levels by 72 %, 24 h after incubation at 20 °C under pH 3 and 6 (54). Palmarosa oil also reduced the zearalenone levels by 99 % at pH 4 and 6 and 20 °C (55). 94 % Fumonisin B1 dissapeared after exposure to cinammon oil at concentration of 280 µg/ml and 30 °C (73).
- (iv). Essential oils for edible purposes or domestic use and their constituents are exempted from commercial pesticide registration requirements in several countries (48). Oils with wider use in industry have high availability. Both factors lowers the costs of phytosanitary products based on oil.

Disadvantages: The essential oils also have following problems that needs to be solved:

- (i) **Standarization of the antifungal activity:** Essential oils are mixtures of several compounds sometimes with 60 constituents or more. The composition of an essential depends on several factors including plant genotype and age, climatic conditions, soil, harvesting time and storage conditions (65). Therefore, composition is not the same every year, even if the essential oil is extracted from plants in the same place. Hence reproducibility of the antifungal activity is only possible, if we know which constituents should be always present in the oil and their minimum levels required. It is worthy to note that the following constituents, ordered from more to less active, provide antifungal activity: phenols > alcohols > aldehydes > ketones > ethers > hydrocarbons (10).
- (ii) Essential oils suppress fungal growth in cereals and other foods at doses usually above those required by food preservatives currently available in the market. Their concentrations with effective fungal control often have adverse effects on organoleptic properties of grains and other food products (48). Essential oils from edible aromatic herbs and spices have been extensively tested for antifungal activity against the toxigenic fungi because it is assumed that these oils will be not toxic for humans. However, there are very few reports on their potential adverse secondary effects on humans and animals at the concentrations they show effective antifungal activity (42).

Some essential oils are biocides, while others have fungistatic effects when applied at high concentrations. Edible sources containing essential oils with antifungal activity are: garlic, thyme, oregano, cloves, basil, coriander, lemon peel, bay leaf, ginger, mint and rosemary. Their main constituents are diallyl sulfide-allyl disulfide, thymol, carvacrol, eugenol, methyl caviacol, linalool, limonene, 1,8-cineole, citral, carvone, and 1,8-cineole, respectively (42).

7.2. Phenolic compounds

Phenolic compounds are widely distributed in leaves, fruits, barks and oils of plants. The *trans*-ferulic and *p*-cumaric acids are constituents of the cereal grain pericarps, they block the synthesis of DON, fumonisins and aflatoxins, when applied at low concentrations required than to control fungal growth (56). Nevertheless, several reports indicate that phenolic compounds have moderate *in-vitro* antifungal activity and little suppression effects on toxigenic fungi when applied on cereal grains (50). A more realistic approach is the use of phenolic compounds as chemosensitizing agents. Some phenolic compounds and their derivatives shift the redox homeostasis of the fungal cells to more oxidative stage that enhances the efficacy of co-applied antifungals e.g., salicylaldehyde (SA) a volatile phenolic compound derivative (17). Gene-deletion mutants of yeast *Saccharomyces cerevisiae* reduces their ability to scavenge reactive oxygen species and free radicals (sod1D, cytosolic superoxide dismutase; sod2D, mitochondrial SOD; and glr1D, glutathione reductase) were more sensitive to SA than their wild-type (40). SA triggers an oxidative burst that enhances the effects of antimycin and strobilurin. Chemosensitization has the potential to reduce the doses of commercial antifungals and in some cases overcomes the fungal resistance (17).

8. ANTI-FUNGAL ALLELOCHEMICALS FROM NATIVE ARGENTINIAN PLANTS

8.1. FABACEAE

(i). *Geoffroea decorticans* (Gill.) Burkart (common name: **Chañar**): This tree is native from the Monte and Chaco regions (Figure 3A). Farmers use their leaves, flowers and bark against respiratory diseases (9). The ethanolic extract from its leaves and twigs significantly inhibited the hyphal growth of *Aspergillus flavus*, *A. parasiticus*, *A. nomius* and *Fusarium* species. Its main antifungal constituents are: 5,7,2',3'-tetrahydroxy-4'-methoxy-5'-prenylisoflavanone and 7,2',3'-trihydroxy-4'-methoxy-5'-prenylisoflavanone (Figure 3B), with MIC = 9-21 µg/ml against *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. Clotrimazole was 0.8-3.3 folds more active than the 5-prenylisoflavanones, whereas, sorbic and ascorbic acids were less effective (58). Lethality test on *Artemia salina* indicated that clotrimazole was toxic (80 µg/ml), while both 5-prenylisoflavanones were moderately toxic (250-290 µg/ml). Fungitoxic selective indexes suggest that 5-prenylisoflavanones are safer antifungal agents than clotrimazole (58).

(ii). *Zuccagnia punctata* Cav. (common name: **Falsa jarilla**): It is shrub (Figure 3C) naturally growing in Monte province (36). Its extracts and isolated components have

antiulcer, antibacterial and antifungal activities (59). The ethanolic extracts of its twigs and leaves of *Z. punctata* (TZP) significantly inhibited the *in-vitro* radial hyphal growth of wood rot fungi and cereal grain rot fungi (35,59). TZP also inhibited the fungi causing soybean diseases in microdilution tests, with MIC = 0.1-0.5 µg/ml (71). Spore germination and early hyphal growth were more sensitive to TZP than mycelial growth (50). Six antifungal phenolic compounds were isolated from its leaves: 2',4'-dihydroxychalcone (DC), 2',4'-dihydroxy-3'-methoxychalcone (DMC), 7-hydroxyflavanone, 7-hydroxy-3',4'-dimethoxyflavone, 1-methyl-3-(4'-hydroxyphenyl)-propyl caffeate and 1-methyl-3-(3',4'-dihydroxyphenyl)-propyl caffeate (Figure 6D). DC and DMC showed the strong biocidal and biostatic activity on *Fusarium* species responsible for cereal grain rot, with MIC = 25-100 µg/ml (36). They are natural constituents (mg/kg) in foods, drinks and propolis and have low toxicity against *Artemia salina*. Both chalcones showed synergic interactions when mixed with potassium sorbate, calcium propionate and pyraclostrobin. We have found that TZP, its ethereal fraction (Eet) and the major EEt constituents (DC, DMC and 7-hydroxy-3',4'-dimethoxyflavone) are effective seed protectants and also stimulates the early seedling growth in maize (37).

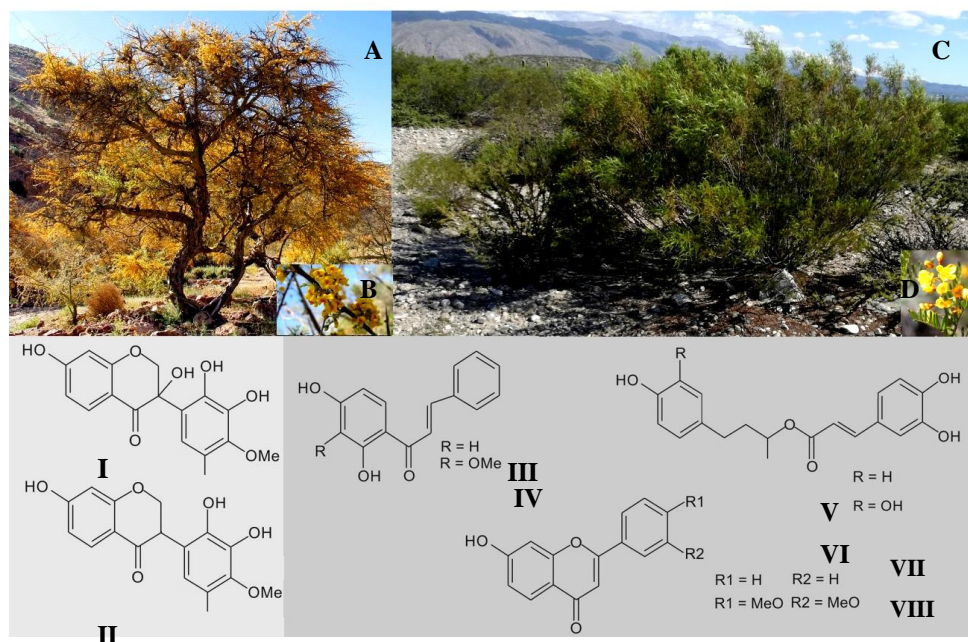


Figure 3. Chañar (*Geoffroea decorticans*) and falsa larrea (*Zuccagnia punctata*): (A,B) tree of *G. decorticans* and its antifungals (I) 5,7,2',3'-tetrahydroxy-4'-methoxy-5'-prenylisoflavanone and (II) 7,2',3'-trihydroxy-4'-methoxy-5'-prenylisoflavanone. (C,D) Shrub of *Z. punctata* and its phenolic compounds (III) 2',4'-dihydroxychalcone, (IV) 2',4'-dihydroxy-3'-methoxychalcone, (V) 7-hydroxyflavanone, (VI) 7-hydroxy-3',4'-dimethoxyflavone, (VII) 1-methyl-3-(4'-hydroxyphenyl)-propyl caffeate and (VIII) 1-methyl-3-(3',4'-dihydroxyphenyl)-propyl caffeate.

(iii). ***Prosopis ruscifolia* Griseb. (common name: Vinal):** It is spiny deciduous tree from the semi arid region of Chaco (Figure 4A)(16) where its monospecific forests make the land unsuitable for agriculture. Its leaves and twigs are traditionally used as antiseptic (34) and their methanolic extract showed wide antifungal spectrum on *Aspergillus* species, with MIC = 750-1500 $\mu\text{g/ml}$ (27). The indolizidine alkaloids juliflorine and juliprosine and the indol alkaloid tryptamine (Figure 4B) were the main antifungal agents of the methanol extract (26). Juliflorine and juliprosine showed strong antifungal effects (MIC = 188 $\mu\text{g/ml}$) while tryptamine had moderate impact (MIC = 750 $\mu\text{g/ml}$) on *A. parasiticus* and *A. flavus*. The methanol extract and its three alkaloids improved the fungitoxic effects of potassium sorbate and propiconazole and completely suppressed the biosynthesis of aflatoxins sublethal concentrations. These alkaloids interact with fungal membrane-embedded proteins. Blocking of calcium channels inhibits the aflatoxin biosynthesis in *A. parasiticus* (57,70), hence, its antiaflatoxigenic activity was observed. These alkaloids have been identified in several *Prosopis* species (27), where they function as phytoanticipins (26).

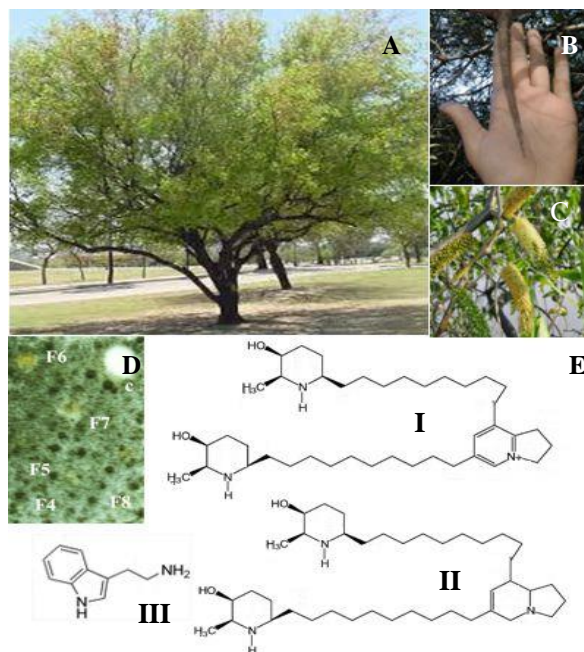


Figure 4. Vinal (*Prosopis ruscifolia*): (A) a general view of tree, (B) stem thorns, (C) Inflorescence, (D) Dot blot bioautography showing inhibition from a fraction of ethanolic leaf extract (F6) and (E) alkaloids identified in F6: (I) juliflorine, (II) juliprosinene and (III) tryptamine.

8.2. ANACARDIACEAE

(i). ***Schinopsis* trees (common name: Quebracho):** *Schinopsis lorentzii*, *S. marginata* (Figure 5A) and *S. balansae* are native from the semiarid region of Chaco (5). Their leaves and bark are used in wound healing and as antiasthmatics (6). In our lab, the

dichloromethane and ethyl acetate extracts from their leaves suppressed the growth of *F. graminearum* ($IC_{50} = 195\text{--}228 \mu\text{g/ml}$) and *F. verticillioides* ($IC_{50} = 252\text{--}490 \mu\text{g/ml}$) (5). The main antifungal constituents of hexane and ethyl acetate extracts were the triterpenoid lupeol and a series 4-alk(en)yl catechols with side chains of 15 and 17 carbon atoms (Figure 5B). Sublethal concentrations of 4-alk(en)ylcatechols and lupeol inhibited the fumonisins and DON biosyntheses of *F. verticillioides* and *F. graminearum*, respectively, which co-occur with a decrease in the fungal production of reactive oxygen species (ROS). The opposite effect was seen, when fungi were subjected to sublethal concentrations of prothioconazole, which increased both ROS and mycotoxin levels. Macroconidia of *F. graminearum* exposed to 4-alk(en)ylcatechols showed an abnormal germ-tube morphogenesis (6). Altogether, these results suggest that the antifungal action mode of 4-alk(en)ylcatechols and lupeol is different from that of prothioconazole.

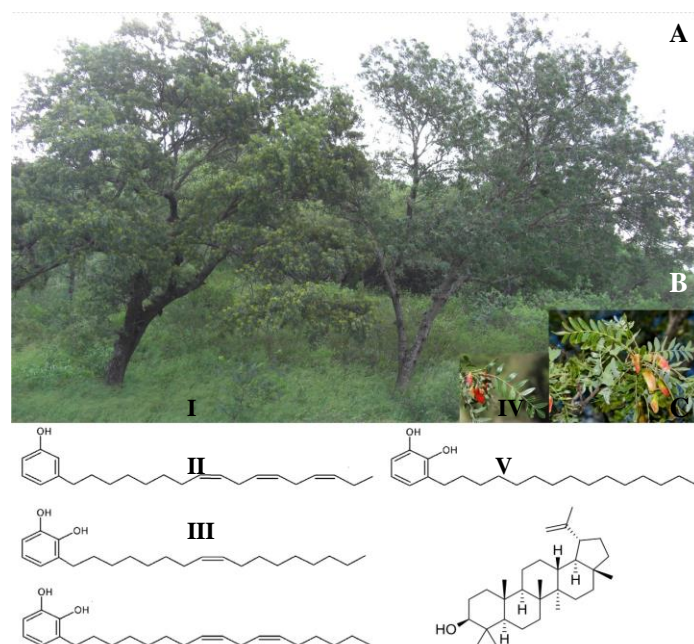


Figure 5. Quebracho colorado (*Schinopsis lorentzii*) and quebracho serrano (*Schinopsis marginata*): (A) A general view of both *S. lorentzii* (left) and *S. marginata* (right) and (B) fruits and leaves in a branch. Compounds contained in their aerial parts are shown in (C) and were (I) 3-heptadec-8,11,14-trienyl phenol, (II) 3-heptadec-8-enyl catechol (III) 3-heptadec-8,11-dienyl catechol, (IV) 3-pentadecyl catechol, (V) lupeol.

(ii). ***Schinus* species (common name: Falsos pimenteros):** It grows in province of Yungas. Whereas, *S. fasciculatus* and *S. areira* are found in provinces of Monte and Chaco (66). Essential oils extracted from leaves and fruits of these plants showed moderate antifungal activity. *S. fasciculatus* oil interacts with epoxiconazole and pyraclostrobin.

8.3. BIGNONACEAE

***Macfadyena cynanchoides* (Cham.) Morong (common name: Sacha gusano):** It is liana from the province of Yungas (Figure 6A). Its dichloromethane stem extract inhibited the growth of *A. carbonarius* and *A. niger* (4), with $IC_{50} = 1.0-1.2$ mg/mL. The main antifungal constituents were the naphthoquinone lapachol (MIC = 0.25-1.00 mg/ml) and 1-hydroxy-4-methylantraquinone (MIC = 0.0625-0.125 mg/mL). Lapachol was also found in the young woody stems of native liana *M. unguis-cati* (Figure 6B). These compounds synergized the antifungal activity of sodium metabisulfite and might be used as additives of commercial antifungals against *A. niger* and *A. carbonarius*.

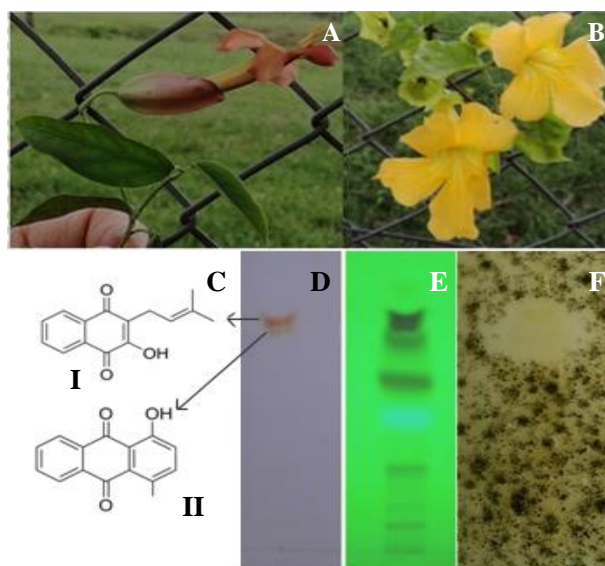


Figure 6. Flowers and leaves of (A) *Macfadyena unguis-cati* and (B) *M. cynanchoides*. Compounds contained in the woody stems of these lianas are shown in (C) and were (I) lapachol (*M. unguis-cati*) and (II) lapachol plus 1-hydroxy-4-methyl anthraquinone (*M. cynanchoides*). These antifungal pigments are visualized in TLC chromatogram (D) as naked eye, (E) under UV and (F) a TLC bioautography against *A. carbonarius*.

8.4. EUPHORBIACEAE

Some native members of *Euphorbia* are used in Argentina against microbial infections (9). Extracts from *Euphorbia* species tested in our lab against *Fusarium graminearum* and *F. verticillioides* allowed the isolation of cycloartenol (CA) and 24-methylcycloartanol (24MCA) from the hexane extract of *E. collina*. These compounds had moderate to weak inhibition of fungal growth (38), but were very effective blockers of deoxynivalenol (DON) and fumonisins biosynthesis. They synergized potassium sorbate (38) and are promising food additives with low mycotoxigenic risk.

9. CONCLUSIONS

This review presented the advances made in the screening of new allelochemicals from wild plants of Northwest Argentina, useful to control grain rot fungi. The antifungals identified were phenolic compounds, alkaloids and terpenoids. Several of them have phytoanticipin role in their producer plant. These metabolites were found in extracts from species of Fabaceae, Anacardiaceae, Bignoniaceae and Euphorbiaceae families. Our findings showed that main features for their use in fungal control are:

- (i) The suppressive activity on fungal growth of most of these compounds yet need to be tested *in-vivo* on cereals under field conditions. Interactions between the allelochemicals and food reduces the allelochemicals bioavailability for fungi. Hence, the *in-vivo* assays are crucial to confirm usefulness of plant antifungals as storage grain protectants.
- (ii) The plant antifungals are less active than commercial fungicides but are also less toxic to untarget organisms. Some of them inhibits fungal growth better than some food preservatives.
- (iii) Their impact on fungal ecophysiology is unknown, especially regarding their mode of action and effects on mycotoxin biosynthesis.

Synergisms reported here suggests that if these compounds are mixed with fungicides, could minimize the fungicides doses in field conditions. Hence, patents based on new antifungal compositions involving antifungal allelochemicals and commercial antifungals are possible. This requires more detailed knowledge to elucidate the specific action mechanism of these allelochemicals, their *in-vivo* effectiveness and other aspects to control *Fusarium* and *Aspergillus* species.

DECLARATION

We declare that all authors of this Ms have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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