Comparison of aqueous extracts of *Citrus×aurantium* and *Aloe vera* L. as fungistatic control of *Fusarium* spp.

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ABSTRACT

Objective: To evaluate the fungistatic capacity of aqueous extracts obtained from *Citrus×aurantium* (sour orange) and *Aloe vera* L. on phytopathogenic fungi of the genus *Fusarium* spp.

Design/Methodology/Approach: Three aqueous extracts were made (aloe peel, aloe peel and gel, and sour orange peel). The inhibition capacity in the in vitro growth of a *Fusarium* spp. strain was measured using the following concentrations: 750 μg/mL, 500 μg/mL, 250 μg/mL, 1 mg/mL, 5 mg/mL, 10 mg/mL, 20 mg/mL, 50 mg/mL, 70 mg/mL, and 100 mg/mL in each extract. The control was sterile distilled water. The radial growth of the mycelium was measured daily, starting from the infected disc up to the edge of its diameter.

Results: The following extracts recorded a good inhibitory response: 100- and 70-mg concentrations of aloe peel and gel in 1 mL of agar; 70-mg concentration of orange in 1 mL of agar; and 100-mg concentration of aloe peel in 1 mL of agar.

Findings/Conclusions: The aqueous extract of aloe had a 47.5% inhibitory potential in the radial growth of *Fusarium*.

Keywords: Aqueous extracts, *Aloe*, *Citrus*, control diseases, *Fusarium*.

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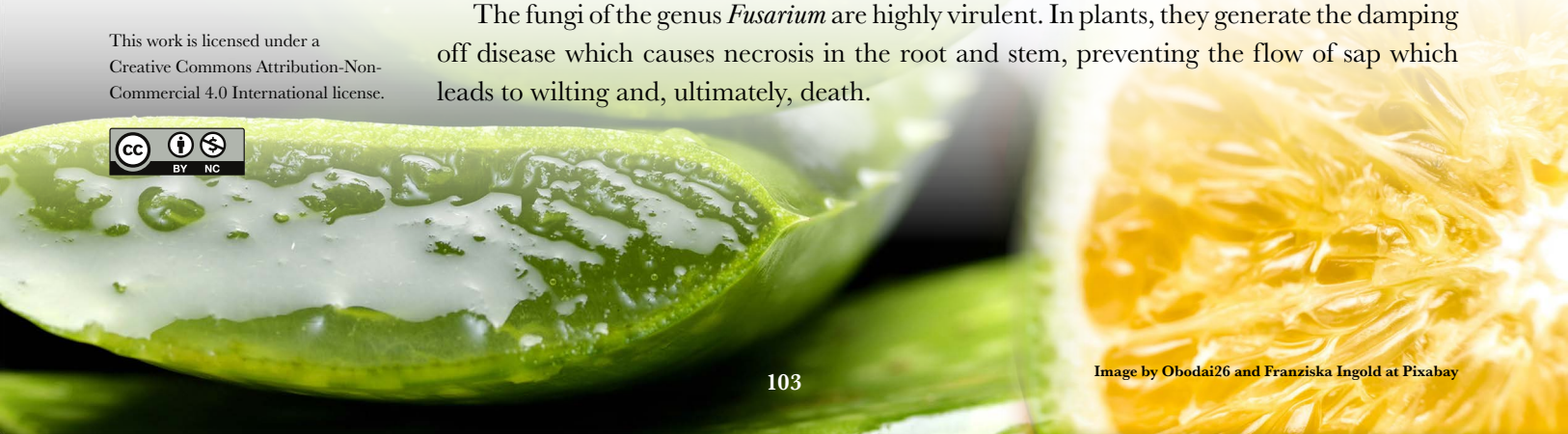
INTRODUCTION

Phytopathogenic fungi are a serious problem from the pre-germination until the post-harvest phase, causing great losses and increasing production costs. Consequently, producers look for means to combat the diseases caused by fungi, applying chemical products that prevent the fungal impact on crops. These chemical products cause soil and groundwater pollution and remove biological controllers from the environment. This phenomenon leads to the presence of more pests and diseases, which consequently increases the consumption of chemical agents used to control pests and diseases, increasing production costs.

The fungi of the genus *Fusarium* are highly virulent. In plants, they generate the damping off disease which causes necrosis in the root and stem, preventing the flow of sap which leads to wilting and, ultimately, death.



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The action of plant extracts on pathogens is related to their secondary metabolites (Marcano and Hasegawa, 2002).

The species of the genus *Citrus* are economically important and have multiple benefits for human health; additionally, they contain different types of polyphenols and aromatic steroidal oils of biological importance (Murphy, 1999).

Aloe vera is chemically characterized by the presence of phenolic compounds (Okamura *et al.* 1996). The gel is mainly made up of water, mucilage (and other carbohydrates), organic acids and salts, enzymes, saponins, tannins, amino acids, traces of alkaloids, vitamins, and various minerals (Reynolds, 2004).

The use of inorganic chemical products affects the soil, subsoil, and fauna, causing the loss of biological controllers, which promotes an increase in pests and diseases in crops (Gan and Wickings, 2017). The problem of phytopathogenic fungi is particularly serious, because they are present throughout the production cycle (from pre-germination to post-harvest). The major genera include: *Botrytis*, *Puccinia*, *Rhizoctonia*, *Cladosporium*, *Fusarium*, *Colletotrichum*, *Aspergillus*, *Alternaria*, *Mycosphaerella*, *Hemileia*, *Tilletia*, *Ceratocystis*, *Cochliobolus*, *Scleptium*, *Sclerotinia*, *Erysiphe*, *Sphaerotheca*, *Phytium*, *Plasmopara*, *Peronospora*, and *Phytophtora*. These fungi reduce the quality and quantity of the product (Hosni *et al.*, 2013; Castaño-Zapata, 2015).

The organic products obtained from plants that are resistant to the fungi that cause damping off can be used as a control alternative. Their production of metabolites inhibits fungal growth. Some of the advantages of organic products over conventional agrochemical products include a lower impact on biotic and abiotic factors; furthermore, they reduce the impact on the natural controllers of the environment, consequently reducing the presence of pests in crops (Sharma and Malik, 2012; Isman and Grieneisen, 2014; Ordanza-Beneitez, 2017).

The objective of this work is to determine the inhibitory potential of sour orange, and aloe extracts and to characterize the secondary metabolites present in the said extracts.

MATERIALS AND METHODS

The test was carried out from August 2018 to June 2019 at the Laboratorio de Biotecnología Vegetal of the Instituto Tecnológico de Chiná, Campeche. Campeche is located in the southeast of the Mexican territory and has a mostly hot sub-humid climate and summer rains (Aw)1. The average annual precipitation is 1,200 mm and the average annual temperature is 27 °C (max. 36 °C and min. 18 °C).

A large quantity of raw material is required to prepare the extracts. Since not enough material can be obtained from dry aloe, fresh plant material was used, given the high water content of the leaves.

Preparation of the extracts

The aloe leaves were collected from the *ejidos* of Chiná and the sour orange fruits were collected at the Instituto Tecnológico de Chiná, Campeche. The extracts were prepared with hexane, ethanol, and sterile distilled water in a 1:3 ratio (m/v).

Extraction

Aloe extract: peel and gel

The aloe leaves were washed with sterile distilled water and cut in pieces of approximately 3.0 cm^3. The aloe pieces (149.50 g) were placed in a 1,000-mL transparent glass bottle (previously filled with 449 mL of ethanol). The bottle was immediately sealed with cling film (Table 1).

The mixture of macerated plant material was in contact with sterile distilled water for 24 h and was constantly stirred. Afterwards, it was filtered using a funnel and cheesecloth to remove plant material. Holes were made in the lids of the bottles to prevent fermentation; they were stored at 5 °C to be used later in tests for the control of *Fusarium*.

Extraction with a rotary evaporator

Extraction of metabolites from plant extracts

This extraction was carried out using a rotary evaporator (Caframo vv 2000) at the Laboratorio de Agua-Suelo-Planta of the Instituto Tecnológico de Chiná.

The aqueous extracts were purified using a 10-mL syringe and a 0.22-μm MILLEX GV filter. The syringe was used to pour 10 mL of extract through the 0.22-μm wide MILLEX GV filter. The content was collected in a 50-mL test tube and refrigerated at 5 °C. This was carried out with each of the three extracts (sour orange peel; aloe peel, and aloe peel and gel).

Phytochemical analysis of plant extracts

The phytochemical analysis was carried out using the methods described by Valencia-Ortíz (1995), in which the steps that must be followed to identify the families of chemical compounds are explained (Table 2).

Evaluation of the inhibition capacity of the extracts

A one μl of sterile distilled water was placed in an Eppendorf tube. Afterwards, an inoculation loop was used to take a sample of *Fusarium* spp., which was introduced in the Eppendorf tube. The tube was stirred vigorously, to guarantee a homogenous spread of the spores, and previously sterilized 6.0-mm diameter filter paper discs were introduced.

For each test, 250 mL of the PDA culture medium were prepared in a 1:25 ratio (m/v) and sterilized in an autoclave at 116 °C for 30 min.

The culture media with the extracts were prepared in a laminar flow hood (Lumina L-120). First the amount of required extract was added in the Petri dish and subsequently the amount of culture medium was added with the following concentrations: 750 μg/μL,

Table 1. Vegetable extracts of *Aloe vera*, and orange applying a 1:3 ratio (m/v), using water as a solvent.

Extract	Weight of the vegetative material (g)	Volume of dissolvent (mL)	Dissolvent
Aloe: peel	129.90	377.70	Water
Aloe: peel and gel	213	639	Water
Sour orange: peel	201	603	Water

Table 2. Methods and compounds determined by the qualitative chemical analysis of the aqueous extracts (Valencia-Ortiz, 1995).

Method	Compound
Shinoda	Flavonoids
Ferric chloride	Phenols
Gelatin	Tannins
Ninhydrin	Amino group
Lieberman-Burchard.	Triterpenes
Börntraguer.	Quinones
Baljet	Lactones
Dragendorff	Alkaloid
Foam	Saponin
Rosemheim.	Leucoanthocyanidins

500 μg/μL, 250 μg/μL, 1 mg/ml, 5 mg/ml, 10 mg/ml, 20 mg/ml, 50 mg/ml, 70 mg/ml, and 100 mg/ml. Afterwards, the media were stirred and the resulting homogeneous mixture was allowed to solidify. The filter paper discs containing the *Fusarium* spp. strains were placed on the solid mixture of PDA with extracts. For each test, a control was added: an additional 1 μL of PDA culture medium was added to a Petri dish, followed by an infected disc of *Fusarium* spp.

RESULTS AND DISCUSSION

Phytochemical analyses were carried out to identify the chemical compounds found in the plant material used. The plant extracts with ethanol, hexane, and water were tested to determine the inhibitory potential of the various concentrations applied to *Fusarium* spp. strains.

Phytochemical analysis of plant extracts

Table 4 shows the secondary metabolites detected in the qualitative chemical analysis carried out in the plant extracts of aloe and orange.

Therefore, the genera *Citrus* and *Aloe vera* contain various chemical compounds that protect them from external attacks by fungi or insects, since they perform various antifungal and antimicrobial activities.

Table 3. Calculation of the extract-agar PDA dilution of the aloe, and orange extracts for the ethanolic and aqueous tests.

# Annex	Titulo
Annex 1	Calculation of extract-agar PDA dilution for the aqueous extract of aloe vera: peel.
Annex 2	Calculation of extract-agar PDA dilution for the aqueous extract of aloe vera: peel and gel.
Annex 3	Calculation of extract-agar PDA dilution for the aqueous extract of sour orang: peel.

Table 4. Qualitative chemical analysis of the plant extracts of aloe and orange.

Metabolite identified	A:P[§]	A:PG[§]	O:P[§]
Saponin	–	+	–
Steroid saponin	+	–	+
Lactones	++	++	+++
Tannins	–	–	
Leucoanthocyanidins	–	–	+
Xanthones and Flavones	+	–	–
Flavonoids	–	–	++
Steroid triterpenes	–	–	+
Amino group	–	–	+++

Metabolite presence: +++ Highest, ++ Higher, + Present, – Null.
[§]Vegetative material: Sour orange: peel (O:P.), Aloe: peel (A:P.), Aloe: peel and gel (A:PG.).

Evaluation of the inhibition capacity of the extracts

Aqueous extracts

Fusarium inhibition tests were carried out using extracts made with sterile distilled water and aloe peel, a mixture of pulp and peel and orange peel. Those extracts could easily be dissolved in the PDA culture medium.

Aloe peel

Figure 1 shows the record of *Fusarium* inhibition after five days of observation using sterile distilled water as solvent.

The inhibitory potential of the extracts of *Aloe vera* on the growth of *Fusarium* was minimal; the 100- and 50-mg concentrations obtained the best results. The best result was obtained by adding 100 mg of extract in agar; in the said treatment, the radial growth was 1.70 cm. The second-best result (1.80 cm) was obtained with 50 mg. In those media in which

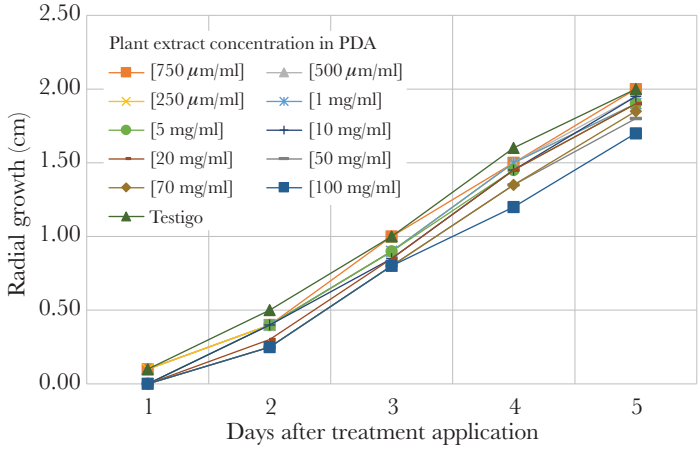

Figure 1. Antifungal activity of the aloe extract (peel) on the radial growth of the mycelium of *Fusarium* spp.

70 mg of extract were added, a growth of 1.85 cm was recorded, while, in concentrations of 20, 5, 1 mg and 250 μg, a radial growth of 1.90 cm was obtained. Conversely, when 10 mg and 500 μg of extract were added, the mycelium reached a 1.95-cm height. Less inhibitory potential was detected in the 700-μg media (2.0 cm); these results are identical to the development of the fungus observed in the control.

Aloe peel and gel

The aloe peel and gel extracts obtained using sterile distilled water as solvent showed a varied behavior on the growth of *Fusarium*. The best results were obtained in the 100-, 70-, and 50-mg concentrations of extract (Figure 2).

The concentration that recorded the best inhibition was 100 mg of extract with a radial growth of 1.05 cm. The 70- and 50-mg concentration did inhibit the development of the fungus to 1.20 cm of radial growth. With the 20-, 10-, 5-, and 1-mg concentrations of extract, the development of the fungus reached 1.80 cm. The 700- and 250-μg concentrations induced a growth of 1.90 cm; this result is similar to the results obtained with the 500-μg concentration (1.95 cm). In the three cases, radial growths were very close to the control (2.0 cm), covering almost the entire petri dish.

Orange

At the end of the inhibition test of the orange extracts on *Fusarium*, a varied radial growth was observed. The concentrations that obtained the best results were 100, 70, and 50 mg of extract (Figure 3).

The concentration that obtained the best inhibition was 100 mg of extract (radial growth: 1.30 cm). The second-best result was obtained in the 70- and 50-mg concentrations, both of which recorded an identical radial growth of 1.50 cm. The 20-, 10-, and 5-mg concentrations obtained a greater radial growth (1.80 cm). For its part, the 1-mg concentration recorded a radial growth of 1.85 cm; this result is similar to the one obtained with 250-μg concentration (1.95 cm). In the 500- and 700-μg concentrations,

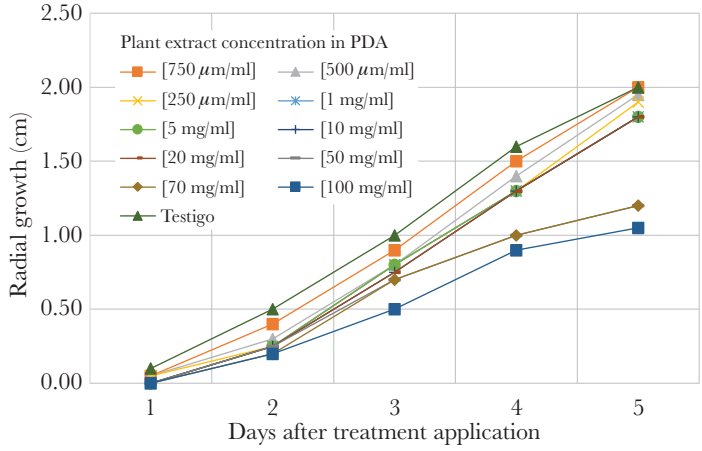

Figure 2. Antifungal activity of the aloe extract (peel and gel) on the radial growth of the mycelium of *Fusarium* spp.

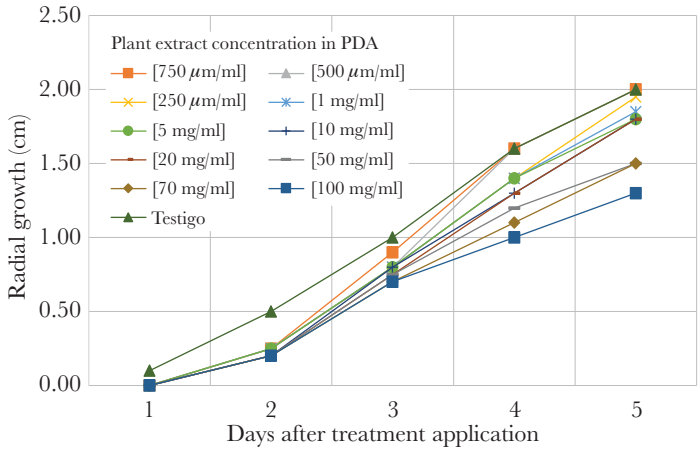

Figure 3. Antifungal activity of the orange extract on the radial growth of the mycelium of *Fusarium* spp.

similar growths (2.00 cm) were obtained, which are identical to those recorded by the control (2.00 cm).

DISCUSSION

The phytochemical analysis of the plant extracts allowed to qualitatively determine the chemical compounds of the peel and gel of the aloe and the peel of oranges. Very high levels were identified in practically all the plant extracts, except for the aqueous extracts of aloe (peel, and peel and gel), which could influence the inhibition of *Fusarium*. In their research, Ruiz and Suarez (2015) mention that the thiol groups of cysteine seem to be the primary targets of sesquiterpene lactones, which causes the inhibition of various cellular functions that lead cells to apoptosis (cell death). In the aloe and orange extracts, the saponin and steroidal saponin chemical compounds could be identified. Ahumada (2016) mentions that the biological properties of saponins include antifungal properties and that their functionality depends on the structural diversity of this metabolite.

Xanthones and flavones were found in the aqueous extract of aloe peel. Reyes *et al* (1997) report that the xanthones were active against *Lenzites trabea*, the fungus responsible for brown rot in wood.

A high presence of flavonols was identified in the orange extracts.

Torres (2017) reports that the *Aloe vera* and moringa extracts affected the development of spore germination (95%) and mycelial growth (68%) of *Colletotrichum* spp., and *Fusarium* spp. Meanwhile, Barkai-Golan (2001) mentions that the evaluated extracts of *Aloe vera* were able to inhibit the spore germination (95%) and mycelial growth (68%) of *Botrytis cinerea*. Finally, Chuchuca (2019) reported that the best alternative to control the crown rot disease caused by fungi in bananas was using 2% *Aloe vera* ethanolic extracts.

CONCLUSIONS

Saponins, lactones, xanthones and flavones were identified in the phytochemical screening of the aqueous extract of aloe peel and gel. These may have an antifungal activity and be responsible for the inhibition of *Fusarium* spp. in the tests carried out.

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