Effect of plant extracts on the inhibition of the mycelial growth of *Penicilium citrinum* Link

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ABSTRACT

Objective: To evaluate in vitro plant extracts of three plant species —mistletoe (*Psittacanthus* spp.), guinea hen weed (*Petiveria alliacea*), and ginger (*Zingiber officinale*)— with the aim of determining their inhibitory effect on the mycelial growth of *Penicilliun citrinum* isolated from coffee beans.

Design/Methodology/Approach: Filtrations were carried out under aseptic conditions using a vacuum system and were added to the Potato Dextrose Agar medium. Once it had solidified, a 5-mm disc of *P. citrinum* was placed in the center of the Petri dish.

Results: The ethyl plant extracts, like the chemical product, showed a 100% inhibition on the pathogen development.

Findings/Conclusions: Ethyl plant extracts can be an agroecological alternative for the control of *P. citrinum*.

Keywords: Mistletoe, guinea hen weed, ginger, antifungal, coffee.

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INTRODUCTION

Coffee (*Coffea arabica* L.) is one of the most important crops in Mexico. Its economic value lies on the generation of foreign currency, it has social value as a generator of jobs and family labor, and its environmental value is mainly the result of its cultivation under shade conditions, the promotion of carbon capture, and the preservation of biodiversity (Escamilla, 2017; CEDRSSA, 2019). In Mexico, 947,092 tons of green coffee are grown in 710,897 hectares. Chiapas is one of the main coffee producing states: 253,457 ha are used to produce 384,549 tons of coffee (SIAP, 2022).

Fungal problems may cause the deterioration of coffee beans in the warehouse; these problems impact the flavor and aroma of the coffee cup. Some species of the genera *Aspergillus*, *Penicillium*, and *Fusarium* have also been related to the production of mycotoxins —toxigenic secondary metabolites that can cause diseases in humans and animals. Ochratoxins and aflatoxins —two of the main toxins that have been reported— are known to have highly carcinogenic effects (Gruber *et al.*, 2017; Garrido *et al.*, 2018; Davidovich *et al.*, 2019).

The use of synthetic fungicides is currently the main method used to control pathogens; however, they can have negative impacts on the environment and also cause health problems to workers and consumers. Likewise, the effectiveness of fungicides can cause resistance

in phytopathogenic microorganisms (Tamilselvi and Arumugam, 2017; Samsidar *et al.*, 2018). Therefore, it is necessary to look for friendlier alternatives (*e.g.*, extracts of plant origin) that are less toxic than pesticides and that do not harm the health of consumers and farm workers (Tamilselvi and Arumugam, 2017; Samsidar *et al.*, 2018). Furthermore, the use of a large number of plants in Mexico can be researched for the control of plant diseases (CONABIO, 1998).

Mistletoe (*Psittacanthus* spp.) is a hemiparasitic plant that is distributed in Mexico and affects numerous fruit and forest species (Balladores, 2017; Castillo, 2018; CONABIO, 2022). Numerous studies have been carried out about the capacity of this plant to control diseases in humans, but to date this capacity has not been tested against phytopathogenic fungi.

Known in Mexico as *hierba de zorillo* ("skunk weed"), due to its content of sulfur compounds, Guinea hen weed (*Petiveria alliacea*) is widely distributed in Mexico, Central, and South America (Sariego 2013; CONABIO, 2022). It has been evaluated for the control of insects, nematodes, bacteria, and fungi of genus *Aspergillus* (Barrera *et al.*, 2017; Bracho *et al.*, 2019; Pinargote *et al.*, 2019; Oredoyin *et al.*, 2020).

Ginger (*Zingiber officinale*) has been used to control various diseases caused in plants by such fungi as: *Moniliophthora roreri* (Cif & Par), *Fusarium verticillioides*, *Aspergillus flavus*, *Penicillium* spp., *Sclerotinia sclerotiorum*, *Sclerotium rlfsii*, and *Colletotrichum gloesporioides* (Santana *et al.*, 2009; Darshana *et al.*, 2014; Joya *et al.*, 2015; Rodrigues *et al.*, 2017; Bracho *et al.*, 2019; Costa *et al.*, 2020; Pérez *et al.*, 2021).

Consequently, extracts of mistletoe (*Psittacanthus* spp.), guinea hen weed (*P. alliacea*), and ginger (*Z. officinale*) were evaluated under in vitro conditions to analyze their inhibitory effect on the mycelial growth of *Penicilliun citrinum* isolated from coffee grains.

MATERIALS AND METHODS

The isolation of *P. citrinum* started with the collection of samples of coffee beans stored in Villaflores, Chiapas, Mexico. The samples were disinfested with 1% sodium hypochlorite for 3 min and placed in dishes with PDA culture medium for 4 d. The *P. citrinum* colonies were transferred and purified. Molecular identification was carried out using the DellaPorta Method (1983) to extract the DNA from monosporic cultures and the Polymerase Chain Reaction (PCR) was performed, using the ITS4 and ITS5 primers (White *et al.*, 1990). The amplified fragments were visualized via agarose gel in TAE. The fragments were purified with the QiaQuick Purification Kit (QiaGene) and submitted for sequencing. The amplified fragment was sequenced and compared with the NCBI (National Center for Biotechnology Information) gene bank database.

Evaluation of extracts from three species

Twelve treatments were evaluated (Figure 1, Table 1). The extracts were obtained from 100 g of leaves and roots of each of the species studied (guinea hen weed, mistletoe, and ginger), which were washed and disinfected with 1% sodium hypochlorite. Subsequently, they were macerated with 1.0 L of water and diluted with 70% alcohol. Then they were placed in flasks at room temperature and stirred for 24 h. Subsequently, they were filtered

Figure 1. Species used for plant extracts. a) Mistletoe, b) Guinea hen weed, c) Ginger.

Table 1. Treatments evaluated (20% plant extracts) for the control of *P. citrinum*.

N.	Treatment
T1	Aqueous plant extracts (PE) of mistletoe flowers
T2	Aqueous PE of mistletoe leaves
T3	Ethyl PE of mistletoe flowers
T4	Aqueous PE of guinea hen weed leaves
T5	Ethyl PE of guinea hen weed roots
T6	Ethyl PE of guinea hen weed leaves
T7	Ethyl PE of mistletoe leaves
T8	Ethyl PE of ginger roots
T9	Aqueous PE of ginger roots
T10	Aqueous PE of guinea hen weed roots
T11	Copper oxychloride
T12	Potato Dextrose Agar

with Whatman® number 4 filter paper and put through a vacuum system using a 0.2-μm milipore syringe filter. The extracts obtained were added to the 20% Potato Dextrose Agar culture medium. Once solidified, a 5-mm disc of *P. citrinum* was placed in the center of the Petri dish. The mycelial growth of *P. citrinum* was measured with a digital vernier, nine days after the start of the experiment (Bell *et al.*, 1982; Gamboa *et al.*, 2003). A completely randomized design with four repetitions was used. The data were subjected to an analysis of variance with the SAS software (version 9.0).

RESULTS AND DISCUSSIONS

Fungus Identification

A non-cottony, bluish green growth with abundant powdery sporulation was observed adhered to the PDA. On the reverse side of the colony, a yellowish-white color was recorded.

Under the microscope, small, globose, unicellular conidia, simple conidiophores, and chains could be seen (Barnett and Hunter, 1998). Regarding the molecular identification, the sequence of the ITS 4 region was compared with the NCBI and showed maximum identity (99.40%) with the KM278105.1 sequence.

The results of the mycelial growth of *P. citrinum* on the ethyl and aqueous plant extracts of mistletoe, guinea hen weed, and ginger at 20% showed that mycelial growth was observed in the aqueous plant extracts; however, a total growth inhibition was reported with ethyl extracts, which shows its viability as a control option (Figure 2). According to the analysis of variance, the results showed no significant differences, neither between the ethyl treatments and copper oxychloride, nor between the aqueous treatments and the PDA. In contrast, there was a significant difference between the aqueous and PDA extracts versus the ethyl extracts and copper oxychloride (Table 2).

According to Tortora *et al.* (2007), Marín *et al.* (2013), and Balladores (2017), *Psittacanthus* contains viscotoxins, flavonoids, coumarins, lecithins, anthraquinones, tannins, triterpenes, alkaloids, polysaccharides, and phenolic compounds. The bacterial activity of the last substance damages the lipids of the plasma membranes, causing the loss of cellular content, which may be related to the results obtained for the growth inhibition of *P. citrinum*.

P. alliacea contains flavonoids, phenols, tannins, saponins, quinones, triterpenes, and alkaloid steroids related to the control of diseases in plants (Montes, 2009; Ochoa *et al.*, 2013). The biological activity of the alkaloids and quaternary ammonium salts found in

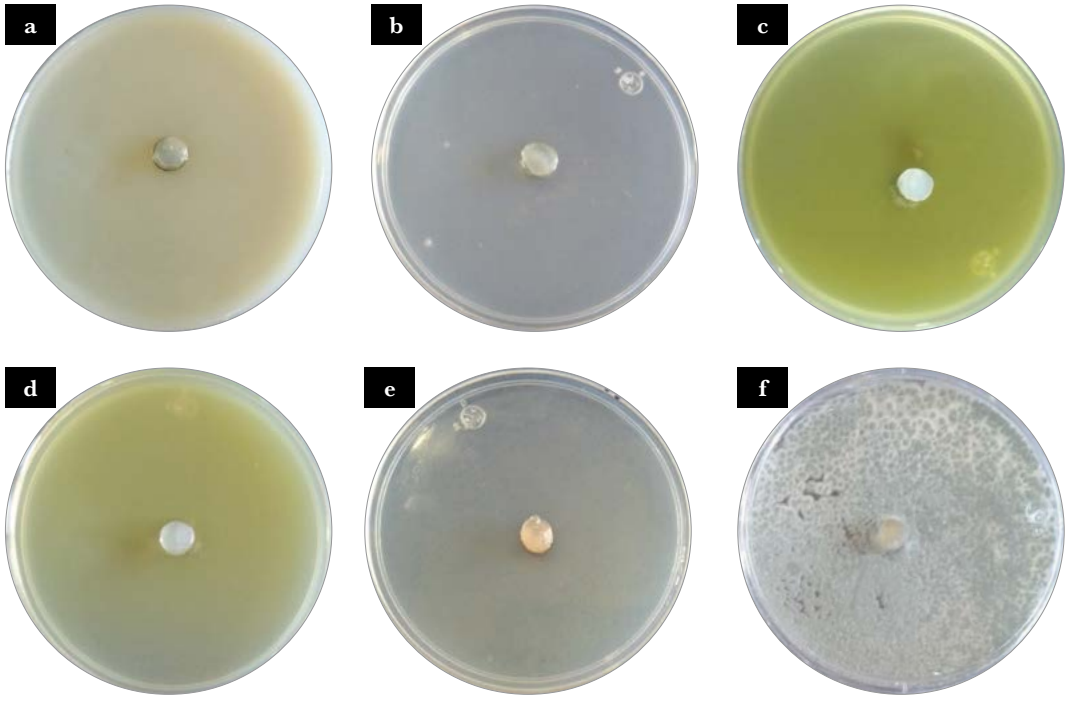

Figure 2. *P. citrinum* in 20% ethyl extracts of mistletoe, guinea hen weed, and ginger: (a) mistletoe flowers, (b) guinea hen weed roots, (c) guinea hen weed leaves, (d) mistletoe leaves, (e) ginger roots, (f) Potato Dextrose Agar.

Table 2. Effect of 20% plant extracts on the mycelial growth of *P. citrinum*.

Treatment	Growth (cm)
T1 Aqueous plant extracts (PE) of mistletoe flowers	7.31±0.58 a
T2 Aqueous PE of mistletoe leaves	6.99±0.74 a
T3 Ethyl PE of mistletoe flowers	0.00±0.00 b
T4 Aqueous PE of guinea hen weed leaves	7.12±0.74 a
T5 Ethyl PE of guinea hen weed roots	0.0 0±0.00 b
T6 Ethyl PE of guinea hen weed leaves	0.00±0.00 b
T7 Ethyl PE of mistletoe leaves	0.00±0.00 b
T8 Ethyl PE of ginger roots	0.00±0.00 b
T9 Aqueous PE of ginger roots	6.68±0.54 a
T10 Aqueous PE of guinea hen weed roots	7.07±0.67 a
T11 Copper oxychloride	0.00±0.00 b
T12 Potato Dextrose Agar	6.55±0.71 a

EV. Vegetable Extract. Means with the same letter are not significantly different, Tukey ($p<0.05$).

ginger helps to control the *Rhizoctonia solani* fungi; additionally, they have a significant participation in the defense of plants against pathogens (Casanova *et al.*, 2004; Him de Fréitez, 2006; Andamayo, 2020). Specifically, as Salch (1997) and Cushnie and Andrew, (2005) also conclude, the plant extracts evaluated contain: flavonoids, phenols, alkaloids, tannins, quinones, and saponin, which seem to be related to the inhibition of the mycelium growth of *P. citrinum*, since they are compounds with high antimicrobial, fungal, antiviral, miticide, and insect repellent activity.

CONCLUSIONS

The 20% ethyl extracts of mistletoe, guinea hen weed, and ginger effectively inhibited the mycelial growth of *P. citrium*. The results of this study indicate that the extracts from the evaluated plant and their parts are taking shape as an effective agroecological alternative for the control of phytopathogens. More detailed studies should be carried out, both regarding biochemical level and extraction methods, in order to determine which molecules and concentrations make up the extracts and which are responsible for antifungal activity against *P. citrinum*. The ultimate aim would be to identify the most effective dosage in the field.

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