Article

Fungistasis of essential oil extracted from a *Tagetes lucida* population of Hidalgo, México

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Abstract

The *T. lucida* species known as 'pericón', is a natural resource of Mexico whose essential oil has antifungal properties, although not all the phytopathogenic fungi have been explored the effect that this vegetable substance can cause. From a population collected in Atotonilco the Grande, Hidalgo, Mexico, essential oil was obtained by hydrodistillation with 0.2% yield; using the CG-EM technique, eight different components were identified in the oil, but the abundant ones were estragole (48%) and anethole (35%). The in vitro antifungal activity of the essential oil against *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium janthinellum* and *Rhizoctonia solani* was also evaluated by means of the agar diffusion technique, performing two experiments: the first testing concentrations of 0, 0.1 and 1% and in the second 0, 2 and 3%. The concentration of 1% reduced the mycelial growth 46% for *F. oxysporum*, 39% in *R. solani*, 21% in *A. niger* and 16% in *P. janthinellum*; however, in high concentrations of oil, such as 3%, only in *R. solani* was the greatest reduction in mycelial growth observed (72%).

Keywords: *Tagetes lucida*, essential oil, antifungal activity, chemical composition.

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Introduction

The genus *Tagetes* (*Asteraceae*) is composed of approximately 56 species distributed throughout the Americas, most of them located in Mexico (Soule, 1996). These plants have biological properties against organisms that are harmful to crops of economic importance: bacteria, fungi, nematodes, insects and weeds (Serrato and Quijano, 1993). Such properties of *Tagetes* are due to the presence of secondary metabolites such as alcohols, ethers, esters, aldehydes, ketones, flavonoids and thiophenes that act as chemical defenses against pests and diseases (Duke, 2008).

In a natural way, these chemical compounds can be released by volatilization, root exudates, leaching and by decomposition of waste (Halbrendt, 1996). Through the use of vegetable material, it has been possible to obtain plant substances such as powders, extracts (aqueous or solvent-based) and essential oils that have been evaluated for their effect against fungi. In the case of some species of *Tagetes*, the plant extracts have antifungal effects, for example, aqueous extracts, powder of leaves, flowers and roots of *T. erecta* have fungicidal effect against *Drechslera oryzae*, *Pyricularia orizae* and *Uromyces phaseoli* (Grainge and Ahmed, 1988) and extracts of leaves and flowers of *T. patula* are fungistatic in *Monilia* spp. (Teodorescu *et al*., 2009); however, there are few toxicological studies, particularly bioassays with essential oils (Serrato-Cruz *et al*., 2007).

Among some phytopathogenic fungi of economic importance, for the damage they cause to various crops, are: *Fusarium oxysporum* (Ascomycete), causing disease in crops such as sorghum (*Sorghum bicolor*), corn (*Zea mays*) and alfalfa (*Medicago sativa*) in its different phenological stages, as well as in fruit and forest trees (Singh *et al*., 2007), *Penicillium* spp. (Ascomycete) causes postharvest rotting of basic grains (Lemmen, 1999); *Aspergillus* spp. (Ascomiceto) in conditions of high humidity in postharvest produce mold in vegetables such as lettuce, tomato and Swiss chard (Raper and Fennell, 1965) and *Rhizoctonia solani* (Basidiomycete) causes seed rot in postharvest of many horticultural species (Anderson, 1982).

As part of the control of these fungi, the use of substances of vegetable origin has been used, among them, the essential oils of onion (*Allium strain*) or garlic (*Allium sativum*) that, evaluated in vitro against *A. niger*, *F. oxysporum* and *Penicillium cyclopium*, no antifungal effect was observed for the first species, but in the others, fungistatic activity occurred (Benkeblia, 2004). Against the same fungi, the essential oil of oregano (*Origanum syriacum*) also produces strong inhibitory action (Daouk *et al*., 1995). So far, the effect of *Tagetes* essential oil against these fungi has not been evaluated, although there are results on other species; for example, *T. patula* oil used against *Botrytis cinerea* and *Penicillium digitatum* under *in vitro* conditions completely inhibits mycelial growth at doses of 10 µL mL^{-1} and 1.25 µL mL^{-1}, respectively.

Refiere (Romagnoli *et al*., 2005), the essential oil of *T. erecta* leaves in 2 µL mL^{-1} completely inhibits the growth of *Pythium aphanidermatum* (Kishore and Dwivedi, 1991) and the oils of *T. minuta* and *T. filifolia* they have antifungal activity against *Sclerotium cepivorum*, *Colletotrichum coccodes* and *Alternaria solani* inhibiting mycelial growth (Zygadlo *et al.*, 1994). Considering that more than 50% of the *Tagetes* species are distributed in Mexico, the realization of studies on possible biological activities against pathogenic organisms in agriculture can be valuable (Serrato-Cruz, 2014).

The species *Tagetes lucida* Cav., Known as 'pericón', is distributed in the main mountain systems of Mexico in temperate and transitional climate (Turner, 1996), it is an herbaceous, perennial plant, up to 1 m high, with a strong smell of anise when squeezed (Villarreal, 2003). The methanolic extract of this plant inhibits the growth of bacteria such as *Escherichia coli*, *Salmonella* sp. and *Shigella* sp., as well as in the species of the *Fusarium* genus due to the action of the dimethoxy-phenolic components present in the extract, such as the scorpion (6,7-dimethoxycoumarin) responsible for presenting a high inhibition on the mycelial growth of *Fusarium* in doses of up to 250 μg mL^{-1} (Cespedes *et al*., 2006).

Other studies, such as that of Barajas *et al*. (2011), show the fungitoxic activity of *T. lucida* oil against the fungi *Monilina fructicola* and *Sclerotium rolfsii* at concentrations of 0.1 and 2 μL mL^{-1}, reducing the production of sclerotia. The presence of methyl eugenol (Bicchi *et al*., 1997) and methyl chavicol or estragole (Marotti *et al*., 2004) have been determined in the essential oil of *T. lucida*, compounds to which different characteristics are attributed with potential be exploited as insecticides or bactericides, and in general, as botanical pesticides (Koul *et al*., 2008). However, the variability of essential oil content of populations of *T. lucida* of Mexico (Serrato-Cruz *et al*., 2007) is unknown and there are few works on their antifungal properties.

The characterization of the essential oil profile of populations of *T. lucida* and its toxicological evaluation against phytopathogenic fungi of economic importance such as *F. oxysporum*, *P. janthinellum*, *A. niger* and *R. solani* is a necessary step for the utilization of local natural resources in organic control strategies for agricultural production. In the state of Hidalgo eight species of *Tagetes* are distributed (Villavicencio *et al*., 2010) and by the climatic characteristics of the entity (INEGI, 2007), the presence of *T. lucida* is important (Serrato-Cruz, 2014). In the present work, the content of essential oil of *T. lucida* from a population of Atotonilco, Hidalgo, Mexico was determined and it's in vitro biological activity against the fungi referred to above was evaluated.

Materials and methods

Vegetal material

On October 26, 2014, the aerial part of plants of *T. lucida* (Figure 1) was collected in the phenological stage of terminal flowering in the town of Tiltepec (coordinates 20° 18' 47" north latitude and 98° 40' 58" longitude west and 2 000 meters above sea level), belonging to the population of Atotonilco the Grande, Hidalgo (Figure 2), of predominant temperate climate semicold C(E)(f) (INEGI, 2007). The conditioned seeds were entered into the Salvador Miranda Colin Germplasm Bank of the Autonomous University of Chapingo with accession code ELL-001-2016, herbarized specimens were also entered into the CHAP Herbarium belonging to the Forest Sciences Division of UACH (voucher 67732). The flower stems were cut into 4 to 5 cm pieces using kitchen knife. From the collection of the material until the completion of the distillation took about 2 hours.

Figure 1. *T. lucida* in flowering (photograph: Edgar López López).

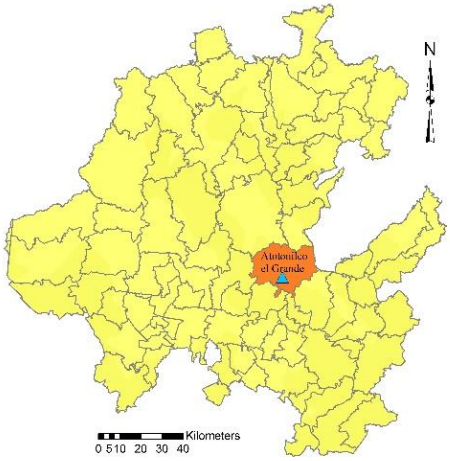

Figure 2. Atotonilco el Grande, Hidalgo and collection point of *T. lucida*. Scale 1: 250 000.

Extraction, oil yield and identification of compounds

The extraction of the essential oil was carried out by hydrodistillation in two modes: laboratory level in an Italian model glass distiller of 6 L capacity (multiple use area in greenhouses of the Institute of Horticulture, in the Autonomous University Chapingo, UACH) and at the pilot level using a stainless-steel distiller with a capacity of 50 kg (pilot plant for the extraction of essential oils in the Experimental Field of Plant Sciences of UACH).

To perform the extraction (repetition) at the laboratory level, 1.5 kg of fresh plant material was used for a distillation with four repetitions, the distillation period was 45 min. For the pilot level extraction 46 kg of fresh material was used, the hydrodistillation time was 3 h from the precipitation. Two phases were obtained from the distillate (aqueous and oily), the oily part was separated and conserved in amber bottles kept in a dark room at a temperature of 18 ±2 °C. The yield of essential oil was obtained in fresh biomass.

For the identification of the components of the essential oil, the oil extract from the pilot distillation was used and analyzed by gas chromatography with mass detector (Adams, 2001). Model CG 7890A chromatograph (Agilent Technologies, USA) coupled to an Inert MSD 5975C selective mass detector with a triple axis detector (Agilent Technologies, USA) with electric impact ionization (IE) of 70 eV. An HP-5ms® column (California, USA), packed with 5% diphenyl-95% dimethylpolysiloxane, was used. The injector and detector temperatures were maintained at 250 and 280 °C, respectively, and were reached at a speed of 10 °C min^{-1}.

The initial temperature of the oven was 70 °C, for 1 min and later it was programmed to reach the temperatures and speed previously indicated. The flow velocity of the carrier gas (helium) was maintained at 1 mL min^{-1}. The samples were diluted (1/100) in acetone (v/v) of 1 µL and subsequently injected in the equipment in automatic "Split" mode by means of a 7683D injector (Agilent Technologies, USA), three repetitions were performed. The data of relative abundance were obtained from the percentage of area of the chromatographic peaks. As major compounds, those with more than 5% relative abundance were considered. The mass range detected was 35-500 m z^{-1}.

The n-alkane compounds, n-octane (C8 H18) and n-octadecane (C18 H38) were used as references in the calculation of the Kovats indices. The identification of the components was made by comparing the relative retention indexes, plus the mass spectra compared with the NIST 05 database of the GC-MS system (National Institute of Standard and Technology) and with the spectral data published by the Carol Stream Corp., USA (Adams, 2001).

Evaluation of antifungal effects

Strains of the fungi *A. niger*, *R. solani*, *F. oxysporum* and *P. janthinellum*, from cultures of the Experimental Agricultural Field of the UACH, were isolated and identified in the Laboratory of Agricultural Mycology of the Department of Parasitology of the UACH. To reproduce each of the strains, from the initial inoculum established in a Petri dish (100 × 15 mm) with PDA medium (200 g potato, 20 g dextrose, 20 g agar), PDA-mycelial sections were made to transfer to fresh culture medium in Petri dishes of the same type, which were incubated at 28 °C for 72 h, then transferred to Petri dishes with each of the treatments, which were bioassayed in two experiments.

Experiment 1. The preparation of the potato dextrose agar plates with the treatments was done as follows. The essential oil in concentrations of 1 mL L^{-1} (0.1%) and 10 mL L^{-1} (1%) was dissolved separately in PDA, to dissolve the oil in the culture medium Tween® 20 was used as emulsifier in 1 mL L^{-1} in all treatments. The commercial fungicide Benomil® (methyl butylcarbamoyl benzimidazol-2-ylcarbamate) at 1% was used as a positive or referential control in addition to including an absolute control without essential oil.

The experiment consisted of 16 treatments generated from the combination of study factors, oil concentration (0.1 and 10 mL), fungal source (four species) and referential control (for each of the fungi), treatments that were distributed following the random block design with four repetitions. Each repetition was confirmed by four experimental units, each experimental unit corresponded to a Petri dish (plate) of 100×15 mm.

From the Petri dishes with the mycelial inoculum agar cuts were made with 1 cm^2 mycelium and they were transferred to the center of the Petri box containing the PDA extract and the respective treatments. Experiment 2. With the same conditions of the first experiment, concentrations of 20 mL L^{-1} (2%) and 30 mL L^{-1} (3%), and Benomil reference (1%) were tested. The experiment consisted of 16 treatments (Table 2), each repeated four times.

In both experiments, the plates or boxes were incubated at a temperature of 28 °C and the mycelial development was observed until the limit growth of the fungus. The activity of the essential oil was evaluated considering the mycelial growth test (Ríos *et al*., 1988); for the recording of mycelial growth, two diameters were measured, which were crossed perpendicularly, adding and dividing in two to calculate the mean growth diameter of the initial inoculum area at 24 h intervals. With the growth diameter data, the percentage of inhibition of mycelial growth with respect to the control treatment (RRT) was calculated by the formula:

$$\text{RRT } (\%)=\frac{\text{TCT-TCt}}{\text{TCT}} \times 100$$

Where: TCT= growth rate in the control and TCt= treatment growth rate.

The analysis of variance on the diameter of the mycelium in the two experiments was done as a factorial 3 × 4 using the general linear model and the comparison of means by the Tukey test ($p \leq 0.05$) with the statistical package SAS version 9 (SAS Institute, 2004).

Results and discussion

Essential oil yield and identification of compounds

The average yield of essential oil at the flask level and at the pilot level was 0.28% with respect to the weight of fresh tissue (Table 1), a result below that reported for this species (0.3 and 0.4%) (Caceres *et al*., 1998) and much lower compared to other species such as fennel (Sellam *et al*., 2015). The characteristics of the habitat in which the population of *T. lucida* develops must surely be important in the response of natural productivity; in this regard, Haslam (1986) proposes that factors such as soil and climate are the main factors that influence the yield and quality of the essential oil.

Table 1. Reference biomass and yield of essential oil of plants of the population of *T. lucida* of Atotonilco el Grande, Hidalgo.

Type	Biomass (kg) fresh weight, PF	Biomass (kg) dry weight, PS	Essential oil extract (mL)	Yield (%) mL 100 g^{-1} PF	Yield (%) mL 100 g^{-1} PS
Flask	1.4075	1.054	4.04	0.2869	0.3832
Pilot	46	35	130.00	0.2826	0.3714
Pilot[z] (1 ha)	2 000	1 513	5 600	0.2800	0.3701

[z]= Extrapolation of the pilot trial to 1 hectare.

Regarding the identification of compounds, nine were determined, separated in retention times of 3.9 to 17 min which were presented in the following sequence: β-myrcene (3.93 min), 3, 7-dimethyl-1, 3, 6- octatriene (4.60 min), 3, 7-dimethyl-1, 6-octadiene-3-ol (5.30 min), estragole (6.67 min), 4-methoxybenzaldehyde (7.43 min), 1-methoxy-4- (1-propenyl)) benzene (7.86 min), 1,2-dimethoxy-4- (2-propenyl) benzene (9.32 min), caryophyllene oxide (11.72 min) and trans-13-octadecanoic acid (17.09) (Table 2, Figure 3).

Table 2. Chemical composition of *T. lucida* essential oil from Atotonilco el Grande, Hidalgo.

Peak	Compound	Retention time (min)	Area	(%)
1	β-mircene	3.93	1 336 863	2.32
2	3,7-dimethyl-1,3,6-octatriene	4.6	425 990	0.74
3	3,7-dimethyl-1,6-octanediene-3-ol	5.3	808 900	1.4
4	Estragol	6.67	28 073 041	48.84
5	4-methoxybenzaldehyde	7.43	742 814	1.29
6	1-methoxy-4- (1-propenyl) benzene (anethole)	7.86	20 569 878	35.78
7	1,2-dimethoxy-4- (2-propenyl) benzene	9.32	1 300 116	2.26
8	Caryophyllene oxide	11.72	836 063	1.45
9	Trans-13-octadecanoic acid	17.09	3 384 380	5.88

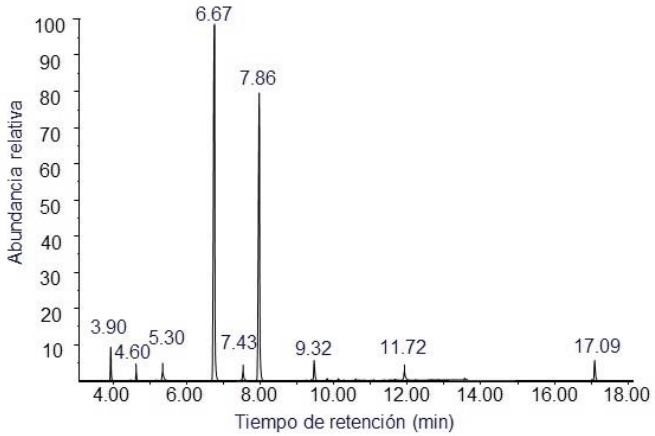

Figure 3. Chromatogram of the components of the essential oil of *T. lucida*.

Evaluation of antifungal activity

The major components were: estragole (1-allyl-4-methoxybenzene) (48%) and anethole (1-methoxy-4- (1-propenyl) benzene) (35%) (Peaks 2 and 4 of Table 2), which have been registered previously for the species in question (Bicchi *et al.*, 1997; Marotti *et al.*, 2004). These chemical compounds are phenylpropanoids that are distinguished by being isomers of position reported in other species of *Tagetes* (Serrato *et al*., 2008), these secondary metabolites have biological effect against various species of insects and fungi (Koul *et al*., 2008; Sellam *et al*., 2015). It should be noted that anethole as a major component combined with other molecules in the essential oil of *T. filifolia,* has a higher biological activity than pure state (Camarillo *et al*., 2009), evidence that

suggests an effect of synergism (Koul *et al*., 2008). Anethole and estragole are found in other aromatic species such as tarragon (*Artemisia dracunculus*), basil (*Ocimum basilicum*), fennel (*Foeniculum vulgare*), anise (*Pimpinella anisum*) and star anise (*Illicium verum*) (Freire *et al.*, 2005; Rietjens *et al.*, 2005).

In both experiments, the morphological characteristics presented by the fungi were as follows. In *A. niger*, the macromorphology of the colonies showed at first a white coloration that turns yellow and after 72 h goes dark brown to black and woolly; *F. oxysporum* showed white colonies at the beginning and later turned purple, of cottony appearance and produced a purple pigment that spread in the culture medium; *P. janthinellum* presented colonies of grayish-green coloration, flat, velvety and fasciculate, as it grew, a remarkable production of exudates, probably secondary metabolites, was noticeable; *R. solani* developed brown and flat brown colonies at the beginning of their growth, later presented cottony white appearance, at the end of its growth, dark green pigmentation was observed. The morphological characteristics of the fungi coincided with that reported for these species in their different phases with limited growth, but without apparent changes (Agrios, 1995).

In experiment 1, the initial diameter of the mycelium in the culture medium with Benomil® remained unchanged at the different recording moments of this variable, that is, there was no significant growth at the end of the evaluation (1.5 mm, $p \leq 0.05$) (Table 3), a response that coincides with other results such as Villa *et al*. (2015) where a fungicidal effect of Benomil® (reference control) is reported with values higher than 95% of activity. On the contrary, in the absolute control, the mycelial growth had the largest diameter (9 mm) (Table 3). The concentration of the essential oil, the fungal species and their interaction influenced ($p \leq 0.05$) the mycelial growth of the phytopathogenic fungi studied (Table 3). Considering the concentration factor of the oil, the diameter of the zone of growth of the fungi was decreasing proportionally when increasing the concentration (Table 3), this effect has been observed in previous works of evaluation of extracts of *Tagetes* in fungi (Céspedes *et al*., 2006).

The tendency observed in the decrease of mycelial growth allows us to suppose that with higher concentrations of oil the total inhibition of growth and perhaps the death of the fungus could be achieved, this consideration motivated the second experiment that will be discussed later. Regarding the growth of the diameter of the mycelium and its relation with the different species of fungi (Table 3), it was found that Ascomycetes *A. niger* and *P. janthinellum* responded in a similar way (8-8.1 mm), with higher growth ($p \leq 0.05$) compared to that of the basidiomycete *R. solani* and the ascomycete *F. oxysporum*, both of similar mycelial growth (6.7-7.1 mm); these differences could be attributed to the genetic nature of these organisms.

Regarding the interaction effect, he highlighted that with 0.1 and 1% of *T. lucida* oil applied to the four-fungal species, a lower mycelial diameter ($p \leq 0.05$) was achieved with respect to the absolute control (Table 3), confirming the effect antifungal of the oil. It was also noted that with 0.1% oil, the diameter of the mycelium of *F. oxysporum* and *R. solani* was lower ($p \leq 0.05$) (4.86.8 mm) than that of *A. niger* and *P. janthinellum* (7.4- 7.9 mm) (Table 3), a response that confirms a certain specificity between the type of fungus and the chemical nature of the oil. In this regard, fungitoxic agents in ascomycetes prevent the growth of the fungus *in vitro* conditions, affect its ability to sporulate, and the germination of conidia (Castellanos *et al*., 2011), in basidiomycete fungi, the

mechanism of antifungal action is given by compounds flavonoids with a biocidal effect (Céspedes *et al*., 2006). Finally, in each fungus species the two concentrations of oil did not influence the diameter of the mycelium with statistical significance (Table 3), a result that would have favorable economic implications of applying the 0.1% concentration.

Table 3. Antifungal effect of *T. lucida* essential oil on the mycelial growth of *A. niger, F. oxysporum, P. janthinellum* and *R. solani* in vitro for 15 days. Factorial experiment 1.

T. lucida oil (%) and control	Diameter (mm) and inhibition of mycelial growth (%)				
	A. niger	*F. oxysporum*	*P. janthinellum*	*R. solani*	Oil factor (C)
	Comparison of averages of factor interaction (HxC)				
0	9.1 ±0.2 a[Z] (0)	9 ±1.1 a (0)	9.1 ±0.3 a (0)	9.1 ±0.5 a (0)	9 ±0.5 A (0)
0.1	7.9 ±0.8 b (13.1)	6.3 ±1.2 c (30)	7.8 ±0.9 b (14.2)	6.8 ±0.6 c (25.2)	7.2 ±0.9 B (20.6)
1	7.1 ±1.1 bc (21.9)	4.8 ±0.2 cd (46.6)	7.4 ±0.1 bc (16.6)	5.5 ±0.7 c (39.5)	6.2 ±0.6 C (31.1)
Species factor (H)	8 ±0.4 A (11.6)	6.7 ±0.8 B (25.5)	8.1 ±0.5 A (10.2)	7.1 ±0.6 B (21.5)	
Benomil® 1	1.5 ±0.5 d (83.5)	1.4 ±0.6 d (84.4)	1.5 ±0.8 d (83.5)	1.5 ±0.3 d (83.5)	1.5 ±0.5 D (83.7)

[Z]= Values with the same lowercase or uppercase letter within the column or uppercase within row are statistically equal (Tukey, $p \leq 0.05$).

The reduction of the mycelial growth rate of the four fungi exposed to 'pericón' oil from Atotonilco the Grande, from 23 to 46% (Table 3), is an indicator of a fungistatic effect of this plant extract containing eight different chemical compounds, with an abundance of estragole and anethole (Table 2). This fungistatic effect caused by *T. lucida* oil had previously been detected against *Sclerotium rolfsii* and *Monilinia fructicola*, important fungi in post-harvest of agricultural products (Barajas *et al*., 2011). For ascomycetes (*A. niger* and *P. janthinellum*), the mycelial reduction rate of 13 to 21%, compared with that recorded for the basidiomycete *R. solani* (25 to 39%) and the ascomycete *F. oxysporum* (30 a 46%), is an indicator that the biological activity of the oil of the Atotonilco population at the concentrations tested does not seem to have promising results against the ascomycetes in this experiment.

In experiment 2, the mycelium in the culture medium with Benomil® did not show a significant increase (1.5 mm) ($p \leq 0.05$) at the end of the experiment (Table 4), while the mycelium of the absolute control had the largest diameter (9 mm, Table 3). The size of the mycelium of the phytopathogenic fungi, as in Experiment 1, was influenced ($p \leq 0.05$) by the fungal species (Table 4): the non-sporulating phytopathogens (*F. oxysporum* and *R. solani*) were less affected than the sporulants (*A. niger* and *P. janthinellum*). On the other hand, there was no concentration effect on the mycelial diameter although this was relatively lower compared to that observed in the first experiment (Table 4).

Table 4. Antifungal effect of *T. lucida* essential oil on the mycelial growth of *A. niger, F. oxysporum, P. janthinellum* and *R. solani* in vitro for 15 days. Factorial experiment 2.

T. lucida oil (%) and control	Diameter (mm) and inhibition of mycelial growth (%)				
	A. niger	*F. oxysporum*	*P. janthinellum*	*R. solani*	Oil factor (C)
	Comparison of averages of factor interaction (HxC)				
0	9 ±0.3 a (0)	9.1 ±0.4 a (0)	8.9 ±0.4 a (0)	9.1 ±0.5 a (0)	9 ±0.4 A (0)
2	6.6 ±0.6 b (26.6)	4.7 ±0.2 c (48.3)	6.8 ±0.6 b (23.6)	3.9 ±0.6 c (57.1)	5.5 ±0.5 B (38.9)
3	6.4 ±0.7 b (28.8)	4.2 ±0.6 c (53.8)	6.7 ±0.3 b (24.7)	2.8 ±0.5 cd (72.5)	5 ±0.5 B (44.9)
Species factor (H)	7.3 ±0.5 A (18.4)	6 ±0.4 B (34)	7.5 ±0.4 A (16.1)	5.3 ±0.5 C (43.2)	
Benomil® 1**	1.6 ±0.6 d (82.2)	1.4 ±0.5 d (84.6)	1.6 ±0.3 d (82)	1.5 ±0.1 d (83.5)	1.5 ±0.4 C (83.7)

[z]= Values with the same lowercase or uppercase letter within the column or uppercase within row are statistically equal (Tukey, $p \leq 0.05$).

Considering the interaction effect, it was observed that 2 and 3% of oil of *T. lucida* presents smaller diameter and higher percentage of mycelial reduction in *F. oxysporum* (4.7-4.2 mm, 48 and 53%) and in *R. solani* (3.9 -2.8 mm, 57 and 72%), with respect to the absolute control (9-9.1 mm) and in comparison, with *A. niger* (6.6-6.4 mm, 26-28%) and *P. janthinellum* (6.8-6.7; 23-24%) (Table 4). These results coincide partially with that reported by Castellanos *et al*. (2011) who indicate that the mycelium of *R. solani* is inhibited by methanolic extracts of *T. lucida* in a dose of 240 μg mL^{-1}. The low inhibition of *A. niger* and *P. janthinellum* species is due to their sporulating activity, since conidia may be more resistant to some antifungal agents (Barajas *et al*., 2011); therefore, the biological activity of the oil of this population of *T. lucida* in the studied ascomycetes is not promising, as previously indicated.

In general, phenylpropanoids such as estragole and anethole, which are found in the essential oil of the Atotonilco population, are secondary metabolites that are also present in other plant species such as fennel (*Foeniculum* spp.) Or anise (*Pimpinela anisum*). Oil has fungistatic or fungicidal activity (Freire *et al.*, 2005; Rietjens *et al.*, 2005; Sellam *et al*., 2015). In the case of the biological activity of Benomil®, the mechanism is given by the inhibitory action of ergosterol synthesis, which is a structural component of the fungal membrane, causing deformation and abnormal proliferation of germinative tubes (Purez *et al*., 1983).

The application of synthetic or natural substances against phytopathogenic fungi should evaluate the implications to the environment and the human, as well as its technical-economic viability, in this last sense, the extrapolation of the biomass and oil yield of the population of *T. lucida* in dry conditions in Atotonilco, Hidalgo, as well as the outstanding fungistatic effect evaluated against the fungi *F. oxysporum* and *R. solani*, allow estimating that with a yield of 2 000 kg of fresh biomass per hectare obtained by harvesting in the natural areas of Atotonilco and with an essential oil yield of 0.28%, approximately 5.6 L of essential oil could be obtained (Table 1), which could be used to formulate more than 1000 L of biopesticide at 1% and more than 10 000 L at 0.1% .

Therefore, this natural resource of Atotonilco, Hidalgo can have good prospects in ecological agriculture against such fungi; its evaluation in the field will be convenient, at the same time as promoting the agroecological management of the 'pericón' population to increase the number of plants per unit area.

Conclusions

The productivity in essential oil of the natural population of *T. lucida* of Atotonilco, Hidalgo was not high and the major components in the oil were anethole and estragole. In vitro evaluation tests against the bioengaged phytopathogenic fungi showed a fungistatic effect of the oil, especially against the fungus *R. solani* in the high oil concentration condition.

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