

In vitro evaluation of the inhibitory activity of essential oils from *Lippia organoides* H.B.K. on mycelial growth and sclerotial production of *Sclerotium cepivorum* Berk.

Evaluación in vitro de la actividad inhibitoria de aceites esenciales de *Lippia organoides* H.B.K. sobre el desarrollo micelial y la formación de esclerocios de *Sclerotium cepivorum* Berk.

Daniel I. Ospina¹, Vanessa Alvarez¹, Harlen G. Torres², Manuel S. Sánchez¹, and Carmen R. Bonilla^{3*}

¹Department of Agricultural Sciences, Universidad Nacional de Colombia, A.A. 237, Palmira, Valle del Cauca, Colombia.

²Department of Engineering and Management, Universidad Nacional de Colombia, Palmira, Valle del Cauca, Colombia.

³Department of Agronomy, Universidad Nacional de Colombia, Bogotá, Cundinamarca, Colombia.

*Corresponding author: crbonillac@unal.edu.co

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Abstract

Essential oils obtained from leaves and flowers of different samples of *Lippia organoides* cultivated in Valle del Cauca, Colombia, were tested in vitro against mycelial growth and sclerotial formation in *Sclerotium cepivorum*, the pathogen responsible for onion white rot. All the evaluated oils in concentrations ranging from 250 to 1350 µL/L exhibited inhibitory activity against both the mycelium and sclerotia of the pathogen. However, those corresponding to the chemotype I (minimum inhibitory concentration, MIC = 120 µL/L) reached the strongest inhibition results regarding the evaluated pathogen structures. Hence, the chemotype I of *L. organoides* can be said to have potential inhibitory activity against onion white rot.

Key words: Antifungal activity, essential oils, *Lippia organoides*, onion, *Sclerotium cepivorum*.

Resumen

Se evaluó la capacidad in vitro de aceites esenciales obtenidos de hojas y flores de diferentes muestras de *Lippia organoides* cultivada en condiciones del Valle del Cauca, Colombia, para inhibir el crecimiento micelial y la formación de esclerocios de *Sclerotium cepivorum*, patógeno causante de la pudrición blanca en cebolla. Todos los aceites evaluados mostraron efecto inhibitorio tanto en el crecimiento micelial como en la formación de esclerocios, en concentraciones de 250 - 1350 µL/L, no obstante, aquellos obtenidos a partir del quimiotipo I presentaron el mayor poder inhibitorio en el crecimiento del micelio (concentración mínima inhibitoria, CMI = 120 µL/L) y en la formación de esclerocios. Así, el quimiotipo I de *L. organoides* puede ser utilizado potencialmente para el control de la pudrición blanca en cebolla.

Palabras clave: Aceites esenciales, actividad antifúngica, cebolla, *Lippia organoides*, *Sclerotium cepivorum*.

Introduction

Generation of products from biological origin is seen as a strategy for pest and disease control in frame with innovative practices for a sustainable and competitive agriculture. Moreover, it is one of the strategies to achieve competitiveness in the agricultural production and marketing at the national level. Research, development, design and formulation of fungistatic products from biological origin are strategies with a positive impact on the environment and health of farmers and consumers. Additionally, these products favor vegetable and fruit marketing abroad, since international markets have strict laws about trace amounts of agrochemicals.

Biologically active compounds from natural sources are interesting to researchers working on infectious disease control on both, humans and plants (Clark and Hufford, 1993). In the last years, the interest on plant extracts with antimicrobial activity has raised, especially on those extracts with potential activity against plant pathogens in vegetable crops, like *Sclerotium cepivorum* Berk., which causes the white stem rot in bulb onion (Pinto *et al.*, 1998; Smolinska and Horbowicz, 1999). In Colombia, this disease has been registered on cold climate production areas, such as Nariño, Boyacá and Tenerife (Valle del Cauca). According to the Ministry of Agriculture and Rural Development of Colombia (MADR) in 2003 Colombia had a bulb onion production area of 11,020 ha, the production was 231,632 t and the partial productivity of the land was 21.69 t/ha, which is higher than the world average (17.45 t/ha). Production is concentrated on nine departments highlighting Boyacá as the highest producer with the best yield per hectare (Melo *et al.*, 2006).

Lippia (Verbenaceae) genus comprises 200 species of grasses and bushes. These species are distributed in Central America (Mexico, Guatemala, Cuba) and South America (Venezuela, Brazil, Colombia). Many of these plants are used in infusion to control gastrointestinal and respiratory diseases and, their leaves are used to season food (Pascual *et al.*, 2001). In South America they are used against cold, flu, bronchitis, cough and asthma. In the case of *Lippia organoides* H.B.K., it is used as condiment for food, as

infusion to treat diarrhea, as analgesic, anti-inflammatory and antipyretic (Morton, 1981) and for its properties as antioxidant, antiseptic, painkiller, cicatrizant, digestive and stimulant (Terblanché and Kornelius, 1996).

Since essential oils (EO) from *L. organoides* have antimicrobial properties (Dos Santos *et al.*, 2004), the objective of the present study was to evaluate its effect on the inhibition of mycelial growth and sclerotial production of the fungi *S. cepivorum* in bulb onion, an important produce of Colombian agriculture.

Materials and methods

Samples used in this study were taken from adult plants of *L. organoides* grown in the Experimental Center of the Universidad Nacional de Colombia in Palmira (CEUNP), where the work Collection of Native Medicinal Plants is located. The original accessions are coming from a recollection done in 2003 in Jordan Sube (canyon basin of the Chicamocha river, Santander, Colombia).

Extraction of essential oils (EO) was done in 200 g of fresh tissue (leaves and flowers) subjected to 3 h of stem distillation. After extraction, anhydrous sodium sulfate was used to remove water. Essential oils were kept in dark vials and refrigerated until their further use in antifungal activity experiments (Gutiérrez *et al.*, 2009). Concentrations were expressed as volume of EO/volume of Petri dish.

Antifungal activity determination

Samples of *S. cepivorum* were collected in Rancho Alegre farm located in Tenerife, Palmira, Valle del Cauca, from plant tissue of infested onion (bulb, leaves, roots) and soil. Sclerotia iso-lated were disinfected with 1% sodium hypochlorite and 70% ethanol, then they were submerged in distilled water for one minute and sown on petri dishes with PDA. Successive sowings were done to obtain clean cultures which were used for the antifungal evaluation of the EO (Jones *et al.*, 2004).

Antifungal activity was determined based on the evaluation of mycelial growth by the agar dilution method (Sahin *et al.*, 2004). In the center of each petri dish a mycelial disc of 5 mm diameter was sown with 20 mL of PDA (potato-dextrose-agar). At least five concen-

trations of EO diluted in Tween-20 were added, they were between 1350 µl/l and 15 µl/l. A control treatment was added as well. Petri dishes were sealed and incubated for four days. Mycelial diameter was scored daily in EO treatments and control until mycelia in the control treatment reached the size of the petri dish. Sclerotia counts were done at the end of three weeks of fungi incubation. Inhibition percentage for mycelia and sclerotia was calculated based on the equation proposed by Manici *et al.* (1997):

$$\text{Inhibición (\%)} = [1 - (\text{Diámetro micelio tratado} / \text{Diámetro micelio control})] \times 100$$

$$\text{Inhibición (\%)} = [1 - (\text{Número esclerocios tratamiento} / \text{Número esclerocios control})] \times 100$$

EO until a concentration of 650 µl/l totally inhibited mycelial growth. From that concentration on lower concentrations were evaluated until the minimum inhibitory concentration (MIC) at which there is not significant mycelial growth was found. Treatments were applied in a completely randomized design with four replications. Data were processed with the Statistical Analysis System software (SAS, 1990) using the Duncan multiple test range to compare between treatments.

Results and discussion

All evaluated EO showed capacity to inhibit mycelial growth of *S. cepivorum* in a concentration range from 250 to 1350 µl/l, with exception of the oils from sample III and from leaves of sample II (Table 1, Picture 1). EO

from sample I both from flowers and leaves had the highest inhibitory activity, MIC of 120 µl/l. Reduction in EO concentrations in all the treatments showed a lower inhibitory effect.

Sclerotia formation process (sclerotia are *S. cepivorum* structures of asexual reproduction that can stay in the soil in a latent stage for 20 years) was completely inhibited by EO of sample I and flowers EO of sample II. Leaves and flowers EO of sample III and flowers EO of sample II allow mycelial growth until sclerotia formation, showing a lower inhibition percentage (Table 2).

EO chemical composition analyzed by gas chromatography coupled to mass spectrometry, showed a different chemical profile for each sample evaluated (Table 3), which is in agreement with preliminary results from Potes (2007). In leaves and flowers of sample I, thymol – a compound with proven anti-fungal action (Braga *et al.*, 2007) – was the main component showing a really high proportion in comparison with the other two samples. This explains its high inhibitory activity for both, mycelial growth and sclerotia formation.

A study of monoterpene activity on mycelial growth (Lucini *et al.*, 2006) shows that borneol, camphor, 1,8-cyneol (eucalyptol), geraniol, linalool, menthol and thymol had MICs of 400, 1200, 1200, 400, 700, 500 and 400 µl/l, respectively. These results demonstrate the inhibitory activity of the EO evaluated in this study and suggest that such

Table 1. Inhibition percentages of mycelial growth in vitro by essential oils (EO) of *Lippia origanoides* in different concentrations.

Sample		EO concentracions (µl/l)				
		1350	650	250	120	60
		Inhibition (%)				
I	Leaves	100	100	100	100	69 c
	Flowers	100	100	100	100	73 cb
II	Leaves	100	77 b*	—	—	—
	Flowers	100	100 a	—	—	—
III	Leaves	94	72	—	—	—
	Flowers	93	73	—	—	—

*Values with the same letter in each simple and column do not show significant differences



Picture 1. From left to right, respectively: Mycelial growth in control, effect of *Lippia organoides*' essential oils on mycelial growth and, mycelial development.

Table 2. Inhibition percentage of sclerotia formation in vitro by the application of essential oils (EO) of *Lippia organoides*.

Sample		EO concentrations range (µl/l)			
		1350	650	250	120
		Inhibition (%)			
I	Leaves	100	100	100	100
	Flowers	100	100	100	100
II	Leaves	100	76 c	—	—
	Flowers	100	100 a	—	—
III	Leaves	66 c*	16 d	—	—
	Flowers	100 a	91 b	—	—

*Values with the same letter in each simple and column do not show significant differences (P < 0.05)

Table 3. Main chemical components in essential oils of *Lippia organoides*.

Sample		Main components	Relative abundance (%)
I	Leaves	thymol; γ-terpinene; p-cymene	45, 13.5, 10
	Flowers	thymol; γ-terpinene; p-cymene	33.1, 13.8, 8.7
II	Leaves	trans-β-caryophyllene; α- humelene; (β+α)-eudesmol	16.7, 13.2, 8.3
	Flowers	trans-β-caryophyllene; α- humelene; (β+α)-eudesmol	17.4, 13, 6.2
III	Leaves	trans-caryophyllene; β-myrcene; α- humelene	16.5, 14.2, 9.7
	Flowers	trans-caryophyllene; β-myrcene; α- humelene	16, 11.2, 10.6

activity is not due to only one component but to the synergistic effect of all the EO components.

Conclusions

- Thymol, γ -terpinene and *p*-cymene were identified as main components of *L. origanoides* essential oils samples (chemotypes).
- Essential oils samples from *L. origanoides* that were evaluated showed their potential use to control onion white rot caused by *S. cepivorum*.
- *S. cepivorum* mycelial growth and sclerotia formation were affected by the application of *L. origanoides* essential oils.
- The highest inhibitory activity was found in the essential oils with the highest content of thymol (leaves and flowers of sample I).

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