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Thyme and suico essential oils: promising natural tools for potato common scab control

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ABSTRACT

- Potato common scab is a worldwide disease mainly caused by *Streptomyces scabiei*. It seriously affects potato crops by decreasing tuber quality. Essential oils (EO) are natural products with recognised antimicrobial properties. In this research, the antibacterial activities of thyme, oregano, suico and mint EO against *S. scabiei* were analysed.
- Infected tubers and soil samples were used for bacterial isolation; the obtained isolates were genetically identified. The chemical composition of the EO was determined by GC-MS. The broth microdilution method was used to analyse antibacterial properties of EO.
- Thirty-one bacterial isolates were obtained. The isolate chosen for antibacterial assays was morpho-physiologically and genetically identified as *S. scabiei*. Thyme EO was mainly composed of thymol and o-cymene; suico EO of dihydrotagetone, trans-tagetone and verbenone; oregano EO of trans-sabinene hydrate, thymol and ɣ-terpinene; and mint EO of menthone and menthol. All the EO tested were effective against *S. scabiei*, but thyme and suico EO were the most successful, with a minimum inhibitory concentration of 0.068 $g{\cdot}l^{-1}$ and 0.147 $g{\cdot}l^{-1}$, respectively, and a minimum bactericidal concentration of 0.137 $g{\cdot}l^{-1}$ and 0.147 $g{\cdot}l^{-1}$, respectively. Scanning electron microscopy showed similar damage caused by both thyme and suico EO to the bacterial envelope. Total phenolic content of EO was not related to their antibacterial activity.
- Thyme and suico EO are effective antibacterial agents against *S. scabiei*, impeding bacterial viability and disturbing the bacterial cell envelope. These EO are promising tools for control of potato common scab.

INTRODUCTION

Globally, potatoes are the third most important food crop after rice and wheat. Potato common scab is a worldwide disease that seriously affects this crop, causing superficial pitted or raised lesions on the tuber surface (Dees & Wanner 2012). These tuber changes reduce the product quality and cause large economic losses (Loria *et al.* 2006; Dees & Wanner 2012). In Argentina, three different pathogenic species have been identified: *Streptomyces scabiei*, *S. acidiscabiei* and *S. turgidiscabiei* (Barrera *et al.* 2013). However, *S. scabiei* is widely described as the causal agent responsible for this disease (Wanner 2006). *Streptomyces* species are nonspecific host pathogens that can infect other crops, such as carrot, radish and beet (Loria *et al.* 2006). The pathogenic *Streptomyces* strains can be found in soil sediment and are spread by wind and water currents or by diseased seed tubers (Wang & Lazarovits 2005).

The infection caused by *Streptomyces* is mainly mediated by the phytotoxin thaxtomin, which acts during the growth stage, altering cell wall formation (Loria *et al.* 2006). Symptom development depends on environmental conditions, host susceptibility, pathogen virulence (Loria *et al.* 2006; Dees & Wanner 2012) and pathogen inoculum density (Dees & Wanner 2012).

At present, several control methods are used: cultural practices (irrigation during tuber growing stage), crop rotation (Loria *et al.* 2006; Dees & Wanner 2012), soil pH reduction, soil fumigation with pentachloronitrobenzene and chloropicrin, organic amendment application, auxin foliar sprays, use of tolerant cultivars (Dees & Wanner 2012) and biological control measures (Meng *et al.* 2013). However, inconsistent and/or inappropriate results have been obtained (Dees & Wanner 2012).

Essential oils (EO) play an important role in plant protection because of their antibacterial, antifungal, antiviral and insecticidal properties (Burt 2004). Their advantages over synthetic pesticides include a mix of active molecules (Bedmutha *et al.* 2011), low mammalian toxicity and non-persistence in fresh water and soil (Isman 2000). Hence, an alternative control based on EO could be appropriate for potato common scab.

Aromatic herbs such as suico (*Tagetes minuta* L., *Asteraceae*), oregano (*Origanum vulgare* L. subsp. *hirtum, Lamiaceae*), thyme (*Thymus vulgaris* L., *Lamiaceae*) and mint (*Mentha* × *piperita* L., *Lamiaceae*) are commonly grown in the province of Córdoba, Argentina. The EO obtained from native and cultivated species from this area have shown antimicrobial properties (Camiletti *et al.* 2014; Asensio *et al.* 2015). Suico is

an annual herb endemic to South America, with Argentina being one of the main producers (Ojeda *et al.* 2015). Oregano, thyme and mint are exotic cultivated plants in Argentina.

The aim of this study was to isolate and identify *Streptomyces* strains that cause potato common scab disease in Cordoba province (Argentina) and to evaluate the antibacterial activities of EO obtained from native and cultivated aromatic plants grown in Córdoba against these bacterial strains.

MATERIAL AND METHODS

Material

The EO were obtained from aromatic plants harvested in Córdoba, Argentina. Leaves and flowers of suico were collected near Pilar, Córdoba Province (31°39′22.32″ S, 63°47′32.11″ W); leaves of oregano and mint were purchased from a farm located in Villa Dolores (Córdoba). Leaves of thyme were obtained from the Experimental Station of the Agronomic Science College (National University of Córdoba). Infected potato tubers (15 samples), harvested in 2016, and soil samples were provided by the Agronomy Science College (National University of Córdoba, Argentina), both were employed in bacterial isolation. A strain of *S. scabiei* was used as a control reference, *S. scabiei* DSM41658^{T} (T-type strain) was purchased from the DSMZ culture collection (Braunschweig, Germany).

Methods

Isolation of Streptomyces spp. from infected tubers and soil
Isolations from scab lesions and soil were performed using the method described by Loria *et al.* (2010) and Lapaz *et al.* (2017). The tubers were washed, surface-disinfected with 1% sodium hypochlorite and rinsed with distilled sterile water. Lesions of each tuber were aseptically removed and ground in saline solution. The homogenate was used to prepare serial dilutions which were spread on nystatin (500 mg·l^{-1}), polymyxin (50 mg·l^{-1}), penicillin (10 mg·l^{-1}), cycloheximide (500 mg·l^{-1}) (hereafter, NPPC water agar), and incubated for 10 days at 28 °C. Isolation from soil was performed using 10 g of a mixture of five soil samples diluted in sterile distilled water. Successive dilutions were prepared and spread on NPPC water agar and incubated at 28 °C for 10 days (Loria *et al.* 2010; Lapaz *et al.* 2017). In both cases, colonies with phenotypic characteristics of *Streptomyces* spp. were transferred onto yeast malt extract agar (YME) (Shirling & Gottlieb 1966) and incubated for 7 days at 28 °C.

Pathogenicity test
A pathogenicity tests on tuber slices were carried out following the method used by Lapaz *et al.* (2017) with some modifications. Tubers were surface-disinfected with 1% sodium hypochlorite and rinsed in sterile distilled water. Slices of tubers (1-cm thick) were obtained aseptically and placed on sterile filter paper discs in Petri dishes. Colonies of the tested *Streptomyces* spp. isolates were incubated for 7 days at 28 °C in YME agar. Plugs of the sporulating colonies were placed inverted onto the tuber slices and were incubated at 28 °C for 5 days in darkness. The test was repeated twice for each chosen isolate.

Extraction of DNA and amplification of txtAB and rpoB genes
Extraction of DNA from *Streptomyces* isolates was performed according to Sambrook & Russell (2001). Isolated DNA was stored at −20 °C until used. Amplification of *txtAB* genes, which are involved in biosynthesis of the phytotoxin thaxtomin A (Healy *et al.* 2002), was carried out using the specific primers TxtAB1/TxtAB2 (Wanner 2006). The amplification of RNA polymerase β subunit (*rpoB*) gene was performed using primer pair SRPOF1/SRPOR1 (Kim *et al.* 2004). PCR analyses were done according to Lapaz *et al.* (2017). The obtained DNA fragments were separated on agarose gel (1.5%) and stained with ethidium bromide under UV light.

Multilocus sequence analysis (MLSA)
The MLSA was conducted on isolates that tested positive in the pathogenicity test and possessed *txtAB* genes. This assay was performed using four housekeeping genes: *atpD* (β subunit of ATP synthase), *recA* (recombinase A), *rpoB* (RNA polymerase β subunit) and *trpB* (tryptophan synthetase subunit B). The PCR mixture and amplification conditions followed Lapaz *et al.* (2017), using the primers described by Guo *et al.* (2008). Amplified DNA fragments were electrophoresed on 1.5% agarose gel and visualised with ethidium bromide under UV light. Isolate(s) identified by MLSA, positive for the pathogenicity test with *txtAB* genes, were subjected to antibacterial tests.

Amplicon purification, sequencing and sequence editing
The PCR products were purified and sequenced in both directions by Macrogen (Kumchunku, Seoul, Korea). The software Contig Express (Vector NTI Advance 10.3; Invitrogen, Carlsbad, CA, USA) was used to edit and assemble the sequences, which were compared with the GenBank database using the BLAST algorithm and deposited into this database (Table 1).

Phylogenetic analyses
A phylogenetic analysis of products obtained by MLSA was conducted using the concatenated sequences of the four genes ordered alphabetically (*atpD*, *recA*, *rpoB* and *trpB*) and later aligned. To perform the analysis, we used the sequences of 28 pathogenic *Streptomyces* isolates from Uruguay described by Lapaz *et al.* (2017) (ST1229, ST1440, ST121, ST103, ST112, ST111, ST105, ST113, ST114, ST116, ST115, ST106, ST107, ST109, ST1020, ST1015, ST1011, ST1013, ST108, ST1018, ST1218, ST119, ST1134, ST124, ST1232, ST1113, ST129, ST127), the sequences of 11 pathogenic *Streptomyces* species deposited in the GenBank database (*S. scabiei* 87.22, *S. acidiscabies* 84-104, *S. europaeiscabiei* NRRL B-24443, *S. luridiscabiei* LMG 21390, *S. niveiscabiei* NRRL B-24457, *S. puniciscabiei* NRRL B-24456, *S. caviscabies* AS 4.1836, *S. reticuliscabiei* NRRL B-24446, *S. stelliscabiei* NRRL B-24447, *S. turgidiscabies* NRRL B-24078 and *S. ipomoeae* NRRL B-12321), and the sequences of the chosen isolates obtained from infected tubers from Argentina. All sequences of each gene were concatenated and aligned using Clustal W in MEGA 5.0 software (Tamura *et al.* 2011). Phylogenetic trees were inferred using the neighbour-joining and maximum parsimony methods (Lapaz *et al.*, 2017), and performed using MEGA 5.0 (Tamura *et al.* 2011). The percentage divergence was calculated between all pairs of sequences based on the alignment. The neighbour-joining tree and topology results were evaluated through 1000 bootstrap re-samplings.

Table 1. Origin and genetic characterisation of *Streptomyces* spp. isolates predicted to cause potato common scab in Córdoba (Argentina).

sample	origin	isolate	type of injury	pathogenicity test[a]	*txtAB*[b]	sequence identification[c]	gene partial sequence	genbank accession
m6	Infected tuber	M6	Deep-pitted	+	–	*Sanguibacter keddieii*	*rpoB*	MK648105
m7	Infected tuber	M7[d]	Deep-pitted	+	+	*Streptomyces scabiei*	*atpDrecArpoBtrpB*	MK648101MK648102MK648103MK648104
m9	Infected tuber	M9	Superficial	+	+	*Streptomyces stelliscabiei/Streptomyces scabiei*	*rpoB*	MK648106
m11	Infected tuber	M11	Deep-pitted	+	+	*Streptomyces stelliscabiei/Streptomyces scabiei*	*rpoB*	MK648107
m12	Infected tuber	M12	Deep-pitted	+	+	*Streptomyces stelliscabiei/Streptomyces scabiei*	*rpoB*	MK648108
m16	Infected tuber	M16	Superficial	+	–	NT		
		M17-2		+	–	NT		
		M17-3		+	+	*Streptomyces* sp.	*rpoB*	MK648109
		M17-4		+	+	*Streptomyces lydicus*	*rpoB*	MK648110
m17	Soil	M17-5		+	+	*Streptomyces* sp.	*rpoB*	MK648111
		M17-6		+	–	NT		
		M17-8		+	–	*Streptomyces pratensis/Streptomyces scabiei*	*rpoB*	MK648112

[a]Isolate pathogenicity: Presence of pathogenicity (+).
[b]Presence (+) or absence (–) of thaxtomin genes.
[c]NT: not tested.
[d]*S. scabiei*[A]: Isolate chosen for MLSA, morpho-physiological characterisation and antibacterial assays.

Morphological and physiological characterisations

The isolate chosen to perform the antimicrobial tests (*S. scabiei*[A]) was morphologically and physiologically characterised according to the International *Streptomyces* Project (ISP) (Loria *et al.* 2010). First, cultures of the tested strain were incubated for 14 days in YME medium to assess colony and spore colour. Water agar was used for spore morphology examination. Tyrosine agar (ISP7 medium) was used to assess melanin production. Raffinose was used as carbon source in ISP9 medium (Shirling & Gottlieb1966) with some modifications: only $FeSO_4.7H_2O$, $(NH_4)_2SO_4$, KH_2PO_4 and distilled water were added to prepare the medium. Dextrose was evaluated as carbon source (positive control). A negative control was prepared with the same medium without the carbon source.

Essential oil extraction and physicochemical characterisation

The EO were extracted from dried plant material (leaves and flowers) by hydro-distillation using a Clevenger-type apparatus, dried over anhydrous sodium sulphate and kept at −20 °C in dark flasks. The EO yields were measured according to Camiletti *et al.* (2014). Their total phenolic content (TPC) was determined using the Folin-Ciocalteu method according to Larrauri *et al.* (2016) and results were expressed as mg gallic acid equivalents (GAE) per g sample.

Analysis with GC-MS and general composition indices

The EO were analysed using a PerkinElmer® gas chromatograph (Clarus 600; Waltham, MA, USA) coupled to a flame ionisation detector (FID) and mass spectrometry detector (MSD), with a capillary column DB-5 (30 m, 0.25 mm i.d., 0.25 mm coating thickness). The carrier gas was helium and the flow rate was 0.9 $ml \cdot min^{-1}$. Ionisation was carried out by electron impact at 70 eV, and mass spectra data were acquired in the scan mode in the m/z range 35–450. The identification of compounds was performed by comparison with published data and mass spectra library NIST (2.0). The quantitative composition was obtained by FID peak area normalisation, using the total peak area as the total composition percentage (100%), and the percentage of each compound related to this. Additionally, the relative abundances of different chemical groups were calculated following the indices performed by Camina (2018): $H = \ln(TM/TS)$, where TM is the relative abundance of EO monoterpenes and TS is the relative abundance of EO sesquiterpenes; $Qm = \ln(HM/OM)$, where HM is the relative abundance of EO monoterpene hydrocarbons, and OM is the relative abundance of EO oxygenated monoterpenes; $Qs = \ln(HS/OS)$, where HS is the relative abundance of EO sesquiterpene hydrocarbons and OS is the relative abundance of EO oxygenated sesquiterpenes.

Essential oil antimicrobial activity

The minimum inhibitory concentrations (MIC) of EO against *S. scabiei* were determined using the broth microdilution method following Carezzano *et al.* (2017), with some modifications. Each strain was inoculated on YME broth and incubated at 28 °C for 72 h. Serial ten-fold dilutions were prepared using the same medium. The appropriate cell density to carry out the MIC assay was determined using a rezasurin sodium salt (Sigma-Aldrich, St. Louis, MO, USA) solution (0.01% w/v) according to Carezzano *et al.* (2017). The dilutions were incubated for 4 h at 28 °C, and the first dilution unable to reduce

resazurin (usually 10^5–10^6 CFU·ml^{-1}) was chosen. A first dilution of each EO was made in dimethylsulfoxide (DMSO), and then nine serial two-fold dilutions were prepared in YME broth. The assay was performed in a sterile 96-well microtitre plate, using columns 1–10 to test the different EO dilutions, while columns 11 and 12 were used as positive control (inoculum) and negative control (assay medium), respectively. The microtitre plate was incubated at 28 °C for 20 h. After that, resazurin solution was added and the microtitre plate was incubated for 4 h at 28 °C. Wells were assessed visually for colour change. The highest dilution remaining blue in colour indicated the MIC.

Additionally, the minimum bactericidal concentration (MBC) was determined as follows: 200 µl of the dilution determined as the MIC and the successive less concentrated dilutions were inoculated in YME agar and incubated at 28 °C for 5 days. The MBC was considered as the highest dilution at which 99.9% final inoculum is killed [NCCLS (National Committee for Clinical Laboratory Standards) 1999].

Scanning electron microscopy (SEM)

The sample preparation for electron microscopy was performed as follows: the selected isolate of *S. scabiei* was cultivated in YME broth containing 0.15% agar for 3 days at 28 °C. The EO at a concentration that showed antibacterial activity were added to YME broth and a final 24 h incubation at 28 °C was performed. Colonies were taken from the medium to be dehydrated with acetone and laminated with gold in Lamarx Laboratories (Facultad de Astronomía, Matemática y Física, National University of Córdoba, Argentina). The *Streptomyces* morphology was examined using a field emission gun SEM (FEG-SEM Carl Zeiss, Oberkochen, Germany) coupled to an In-Lens detector for secondary electrons.

Statistical analysis

The experiments were repeated three times and the results were expressed as mean ± SD. Normal distribution was tested with a Shapiro–Wilk test. ANOVA ($\alpha = 0.05$) and LSD Fisher's multiple range test were performed to determine significant differences between means. Multivariate ANOVA (MANOVA, $\alpha = 0.05$) was performed to analyse significant differences in the relative abundances of different chemical groups in EO. The data were analysed using InfoStat software (Di Rienzo *et al.* 2018).

RESULTS

Isolated Streptomyces *spp., pathogenic strains, genetic and morpho-physiological characterisation*

A total of 31 isolates with typical phenotypic characteristics of *Streptomyces* spp. were obtained from infected potato tubers (superficial, raised or deep-pitted lesions) and soil. Of these, 12 were positive in the pathogenicity test and seven tested positive for the amplification of *txtAB* genes (Table 1). Different species were identified using the *rpoB* sequence in infected tubers and soil samples. Isolates from infected tubers were identified as *S. scabiei*/*S. stelliscabiei* and *Sanguinobacter keddieii*, while the isolates obtained from soil samples were identified as *Streptomyces* sp., *S. lydicus* and *S. pratensis*/*S. scabiei*.

Three potato tuber *Streptomyces* spp. isolates that tested positive for pathogenicity and the presence of *txtAB* genes (Table 1) presented 99–100% similarity to *S. scabiei*/ *S. stelliscabiei* by comparison with the GenBank database using the *rpoB* gene. One of these (M7) was selected to perform antibacterial assays. It was identified as *S. scabiei* (*S. scabiei*^{A}) using MLSA and after constructing a phylogenetic tree, which showed that M7 was strongly associated with *S. scabiei* 87.22 and the isolates ST124, ST1232, ST1113, ST129 and ST127, which have been identified as *S. scabiei* by Lapaz *et al.* (2017). In addition, the morpho-physiological characteristics showed the presence of light brown colonies and light grey spores on YME medium, melanin production on tyrosine agar and visible growth on modified ISP9 medium (with raffinose as carbon source).

Yield, TPC and GC-MS analysis of EO

Leaves and flowers of suico contained 1.12% (v/w) EO (ml EO·100·g^{-1} sample dry weight). Leaves of oregano, thyme and mint contained of 1.03%, 1.00% and 1.47% (v/w) EO, respectively. The highest TPC were found for the EO of oregano (14.02 mgGAE·ml^{-1}) and thyme (13.17 mgGAE·ml^{-1}), followed by those of suico (5.03 mgGAE·ml^{-1}) and mint (1.86 mgGAE·ml^{-1}).

The EO compositions were analysed using GC-MS (Table 2). The EO of thyme was mainly composed of o-cymene (37.1%) and thymol (34.8%), followed by eucalyptol (4.9%), carvacrol (3.8%) and ɣ-terpinene (2.8%). The major compounds of oregano EO were trans-sabinene hydrate (18.6%) and thymol (18.1%), followed by ɣ-terpinene (11.7%), o-cymene (6.8%) and 4-terpineol (6.1%); the major constituents of mint EO were menthol (40.1%), menthone (24.2%) and eucalyptol (8.3%); and the EO of suico was mainly composed of dihydrotagetone (34.8%), verbenone (31.4%), trans-tagetone (11.8%) and β-cis-ocimene (8.8%).

The general composition (Table 2) indicated that the EO were mainly constituted of monoterpenes, predominantly oxygenated, with a small proportion of sesquiterpenes, most of them hydrocarbons. The EO of mint was statistically different from the other EO, with higher amounts of oxygenated monoterpenes and a high relative quantity of sesquiterpene hydrocarbons ($Qm = -3.5$, $Qs = 4.0$). The EO of suico presented the highest proportion of total monoterpenes ($H = 5.0$) but had less oxygenated monoterpenes ($Qm = -2.0$) in comparison with the EO of mint, but a higher amount when compared to the EO of thyme and oregano. The composition of EO of thyme was similar to that of oregano, differing only in the composition of total monoterpenes in relation of total sesquiterpenes ($H = 3.1$ and $H = 2.9$, respectively).

Antimicrobial activity of essential oils

All tested EO presented bacteriostatic and bactericidal activities (Table 3). The EO of thyme and suico had the lowest MIC (0.068 and 0.147 g·l^{-1}, respectively) and MBC (0.137 and 0.147 g·l^{-1}, respectively) (Table 3). These values were followed by EO of oregano and mint (MIC: 0.357 and 0.943 g·l^{-1}, MBC: 0.513 and 1.358 g·l^{-1}, respectively), which had the lowest antibacterial activity.

Table 2. Chemical profile and relative abundance (normalised FID peak area percentage) of the main compounds present in the five essential oils according to the GC–MS analysis.

		relative abundance (%)			
compound	retention time	thyme[a]	mint[a]	oregano[a]	suico[a]
α-Thujene	6.19	0.12		0.84 ± 0.07	
α-Pinene	6.35	0.85 ± 0.04	0.42 ± 0.03	0.57 ± 0.07	
Camphene	6.60	0.41 ± 0.04		0.28 ± 0.03	
1-Octen-3-ol	6.89	0.85 ± 0.04			
β-Pinene	6.98	0.12 ± 0.05	0.65 ± 0.47	3.48 ± 0.28	
β-Myrcene	7.03	0.26 ± 0.02		0.97 ± 0.71	
3-Octanol	7.11	0.07 ± 0.02			
α-Phellandrene	7.35	0.02 ± 0.02		0.17 ± 0.02	
α-Terpinene	7.53	0.55 ± 0.04	0.09 ± 0.01	3.4 ± 0.28	
o-Cymene	7.65	**37.11 ± 1.58**	0.06 ± 0.01	**6.78 ± 0.36**	
β-cis-Ocimene	7.71			4.15 ± 0.36	**8.76 ± 0.4**
Limonene	7.73	0.47 ± 0.19	1.01 ± 0.08		2.31 ± 0.11
β-Phellandrene	7.74			0.82 ± 0.02	
Eucalyptol	7.80	4.89 ± 0.18	**8.28 ± 0.39**		
β-trans-Ocimene	7.84			0.53 ± 0.01	
Dihydrotagetone	7.87				**34.79 ± 1.17**
γ-Terpinene	8.10	2.83 ± 0.17	0.21 ± 0.04	**11.67 ± 0.95**	
3-Carene	8.29	0.35 ± 0.05			
cis-Sabinene-hydrate	8.30		2.17 ± 0.05	3.01 ± 0.17	
α-Pinene oxide	8.47				0.6 ± 0.04
Linalool oxide, (Z)	8.48	0.12 ± 0.03			
Dehydro-p-cymene	8.53	0.13 ± 0.03			
Terpinolene	8.54	0.18 ± 0.04	0.03 ± 0.01	0.73 ± 0.04	
α-Linalool	8.55				0.42 ± 0.07
β-Linalool	8.60	2.59 ± 0.17	0.21 ± 0.03		0.25 ± 0.01
trans-Sabinene-hydrate	8.69		0.13 ± 0.1	**18.61 ± 0.76**	
cis-Tagetone	9.04				0.48 ± 0.02
trans-Tagetone	9.16				**11.8 ± 0.2**
Pinocarveol	9.23	0.06 ± 0.02			
Menthone	9.39		**24.17 ± 0.68**		
Isomenthone	9.49		3.99 ± 0.19		
Neomenthol	9.53		2.63 ± 0.09		
Borneol	9.59	1.11 ± 0.07		1.14 ± 0.1	
Menthol	9.66		**40.12 ± 0.07**		
4-Terpineol	9.67	0.54 ± 0.11		**6.11 ± 0.05**	
4-Isopropenyl toluene	9.71	0.34 ± 0.18			
Isomenthol	9.78		0.57 ± 0.04		
Terpineol	9.82	0.43 ± 0.17	0.45 ± 0.09	2.01 ± 0.08	
Isopiperitone	9.91			0.13 ± 0.02	0.54 ± 0.06
Thymol methyl ether	10.17	0.58 ± 0.04		0.14 ± 0.02	
Carvacrol methyl ether	10.25			1.5 ± 0.04	
Verbenone	10.28				**31.39 ± 0.11**
Pulegone	10.36		0.49 ± 0.06		
Piperitone	10.55		0.59 ± 0.07		
Neomenthyl acetate	10.68		0.12 ± 0.02		
Isothymol	10.78	0.4 ± 0.03			
Menthyl acetate	10.88		3.49 ± 0.33		
Thymol	10.93	**34.83 ± 0.72**		**18.06 ± 0.55**	
Carvacrol	11.01	3.84 ± 0.82		1.83 ± 0.04	
Isomenthyl acetate	11.10		0.08 ± 0.02		
Piperitenone	11.56		2.92 ± 0.35		
Isobornyl propionate	11.94	0.14 ± 0.01			
Copaene	12.00	0.1 ± 0.02			
Farnesane	12.04				0.12 ± 0.01
β-Bourbonene	12.11	0.13 ± 0.02	0.32 ± 0.04	0.15 ± 0.01	
E- Isoeugenol	12.21				0.51 ± 0.1

(continued)

Table 2. (Continued)

compound	retention time	relative abundance (%) thyme[a]	mint[a]	oregano[a]	suico[a]
β- Caryophyllene	12.54	1.12 ± 0.06	2.25 ± 0.16	2.35 ± 0.04	0.19 ± 0.07
β-Farnesene	12.72		0.18 ± 0.04		
α-Humulene	12.96		0.04 ± 0.02	0.08 ± 0.03	0.18
γ-Muurolene	13.19	0.18 ± 0.01	2.01 ± 0.25		
Germacrene D	13.23			1.28 ± 0.06	0.05
α-Amorphene	13.31	0.05 ± 0.01			
γ-Gurjunene	13.41		0.16 ± 0.04		
γ-Cadinene	13.58	0.84 ± 0.04			
δ-Cadinene	13.60		0.05 ± 0.01	0.17 ± 0.03	
Calamenene	13.66	0.24 ± 0.02			
Spathulenol	14.35		0.06 ± 0.04	0.62 ± 0.04	2.87 ± 0.11
Caryophyllene oxide	14.43	1.35 ± 0.12	0.05 ± 0.03	0.32 ± 0.03	0.76 ± 0.05
Cadalene	15.39	0.17 ± 0.02			
Total compounds (%)		98.37	98.73	91.90	96.02
Composition[b]		A	B	A	C
H[c,d]		3.11 ± 0.04[A]	2.90 ± 0.02[B]	2.86 ± 0.04[B]	4.95 ± 0.04[C]
Qm[c,d]		−0.13 ± 0.04[A]	−3.52 ± 0.35[B]	−0.42 ± 0.03[A]	−1.99 ± 0.04[C]
Qs[c,d]		0.74 ± 0.00[A]	3.99 ± 0.37[B]	1.46 ± 0.05[A]	0.63 ± 1.26[A]

[a]Bold numbers denote major compound in essential oil compositions.
[b]Different letters indicate a significant difference at α = 0.05 (MANOVA)
[c]The same letter in a row denotes no significant differences at α = 0.05 (n = 3, LSD Fisher).
[d]H = ln(relative abundance of EO monoterpenes/relative abundance of EO sesquiterpenes), Qm = ln(relative abundance of EO monoterpene hydrocarbons/relative abundance of EO oxygenated monoterpenes) and Qs = ln(relative abundance of EO sesquiterpenes hydrocarbons/relative abundance of EO oxygenated sesquiterpenes).

Scanning electron microscopy (SEM)

To assess the effects of thyme and suico EO on *S. scabiei* morphology, SEM microphotographs were taken. In treatments with these antimicrobial agents, morphological changes of the cell envelope were observed. The control treatment presented vegetative hyphae with a smooth surface (Fig. 1A), while in both EO treatments, considerable alterations to the vegetative hyphae were observed. Regarding the superficial morphology, protrusions and a granular surface were detected (Fig. 1B, 1C). Vegetative hyphal thickness was measured for each treatment and statistically analysed. ANOVA results showed no significant differences in hyphal thickness between treatments ($P = 0.165$), suggesting that the applications of thyme and suico EO may not produce cellular content leakage or disruption of the cell membrane.

DISCUSSION

Isolates from infected tubers were identified as *S. scabiei*/*S. stelliscabiei*, recognised as potato common scab pathogenic agents. Additionally, an isolate identified as *S. keddieii* was obtained. This species was first isolated from cow blood; later, Rahman *et al.* (2018) also found it in the barley seed microbiome. In this case, the presence of *S. keddieii* could be related to the normal diversity of microorganisms associated with scab lesions. An interesting result was the presence of *S. lydicus* in soil samples, which has been described as a common potato scab agent (Kreuze *et al.* 1999). The latter tested positive for pathogenicity

Table 3. Average values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for each tested essential oil against two strains of *S. scabiei*. Values expressed in $g \cdot l^{-1}$.

essential oil	strain[a]	mean ± SD[b]
Suico		
MIC	*S. scabiei*[T]	0.184 ± 0.064[AB]
	S. scabiei[A]	0.147 ± 0.064[AB]
MBC	*S. scabiei*[T]	0.184 ± 0.064[a]
	S. scabiei[A]	0.147 ± 0.064[a]
Thyme		
MIC	*S. scabiei*[T]	0.067 ± 0.044[A]
	S. scabiei[A]	0.068 ± 0.026[A]
MBC	*S. scabiei*[T]	0.095 ± 0.033[a]
	S. scabiei[A]	0.137 ± 0.086[a]
Mint		
MIC	*S. scabiei*[T]	0.757 ± 0.323[B]
	S. scabiei[A]	0.943 ± 0.289[C]
MBC	*S. scabiei*[T]	1.510 ± 0.658[b]
	S. scabiei[A]	1.358 ± 0.510[c]
Origanum		
MIC	*S. scabiei*[T]	0.618 ± 0.441[AB]
	S. scabiei[A]	0.357 ± 0.174[B]
MBC	*S. scabiei*[T]	0.665 ± 0.435[ab]
	S. scabiei[A]	0.513 ± 0.191[b]

[a]T: DSM41658[T], A: *S. scabiei* isolated from potato.
[b]The same letter in a column denotes no significant differences at α = 0.05. Capital letters are related to MIC and small letters are related to MBC.

and the presence of *txtAB* genes; however, it was not isolated from the tuber lesions. The isolate M7, obtained from infected tubers, was genetically identified as *S. scabiei* using MLSA and phylogenetic analysis, and the morpho-physiological characteristics were compatible with *S. scabiei* according to Loria *et al.* (2010). This species was also found by Barrera *et al.* (2013) in soils in the province of Córdoba (Leones, Marcos Juarez) and other areas of Argentina.

For oregano and mint EO, the TPC values were similar to those found by Zaidi & Dahiya (2015) and Dambolena *et al.* (2010). Thyme EO presented a chemical composition similar to that obtained by Lemos *et al.* (2017), who reported thymol, o-cymene and ɣ-terpinene as the main compounds of this EO. Oregano EO was mainly composed of trans-sabinene hydrate, thymol and ɣ-terpinene. A similar composition has been reported by Asensio *et al.* (2015), who analysed the same subspecies.

Mint EO presented menthol and menthone as main compounds, what is in accord with the chemical composition reported by Camiletti *et al.* (2014). Suico EO was mainly composed of dihydrotagetone, verbenone and trans-tagetone, being similar to that obtained for different chemotypes of suico from Argentina by Gil *et al.* (2000). However, Camiletti *et al.* (2014) found verbenone, trans-tagetone and β-cis-ocimene as the main compounds. Such variations in the composition of suico EO could be attributed to genetic differences and environmental factors (Gil *et al.* 2000).

Previous studies have demonstrated antibacterial properties of EO against actinomycetes of importance to human health (Lang & Buchbauer, 2012), especially those whose principal compounds were thymol or carvacrol. Nevertheless, there are few reports of natural products used in the control of *S. scabiei* (Meng *et al.* 2013). The EO of thyme, oregano, suico and mint have previously been recognised as good antibacterial agents against Gram-positive bacteria (Dorman & Deans 2000; De Sousa Guedes *et al.* 2016; dos Santos *et al.* 2017). In our experiments, EO of thyme and suico were the most effective antibacterial agents against *S. scabiei*^{A}.

As previously shown, EO of thyme was mainly composed of thymol and o-cymene. Thymol is a phenolic monoterpene with high antimicrobial activity towards a large range of species (Dorman & Deans 2000; Ultee *et al.* 2002; Burt 2004; Lemos *et al.* 2017). Their hydroxyl group works like a system of delocalised electrons that play an important role in the antimicrobial activity (Ultee *et al.* 2002). In addition, p-cymene has shown variable antibacterial activity (Dorman & Deans 2000; Miladi *et al.* 2017). However, cymene combined with thymol had a synergistic effect against *Bacillus cereus* (Delgado *et al.* 2004). The mechanism of action of these compounds on bacteria appears to be related to membrane expansion, causing destabilisation and decreasing the membrane potential (Ultee *et al* 2002). A previous study has also reported a decrease in pH in presence of carvacrol (most likely, with effects similar to those of thymol), resulting in the absence of a proton motive force and the consequent depletion of ATP pools (Ultee *et al.* 2002). Additionally, components like linalool (Lemos *et al.* 2017) and β-caryophyllene (Bernardes *et al.* 2010), present in smaller amounts in EO of thyme, have also been reported as antibacterial agents contributing to its strong antibacterial activity.

The EO of oregano had a chemical composition similar to that of the EO of thyme, albeit with different quantities of the components. Thymol, carvacrol and o-cymene occurred in lower amounts than in the EO of thyme, which might be related to the lower antimicrobial properties of this EO. Furthermore, higher percentages of sabinene hydrate isomers with no antibacterial activity, according to Matias *et al.* (2016), ($MIC \geq 1.024\ g{\cdot}l^{-1}$) were present in this oil. Compounds present at low concentrations, such as 4-terpineol, eucalyptol (Carson & Riley, 1995) and β-caryophyllene (Bernardes *et al.* 2010), have been recognised as good antimicrobials. Overall, oregano EO could be defined as a good antibacterial agent against *S. scabiei*, but less effective than the EO of thyme and suico.

The EO of suico also possessed powerful antibacterial activity against *S. scabiei* and was mainly composed of ketones (dihydrotagetone, verbenone and trans-tagetone). In a previous study, verbenone was reported to have antibacterial properties against *Staphyloccocus aureus* and *Bacillus subtilis* (Koutsoudaki *et al.* 2005). However, Paraschos *et al.* (2011) obtained a high MIC value for verbenone against a strain of *S. aureus* ($11.1\ g{\cdot}l^{-1}$) and no MBC, suggesting no antibacterial effect. To our knowledge, there are no reports of the antibacterial effects of dihydrotagetone and tagetone. Senatore *et al.* (2004) reported that the EO of suico with high proportions of dihydrotagetone and tagetone has higher antibacterial properties than other chemotypes with low percentages of these compounds. As a consequence, dihydrotagetone, tagetone and, possibly, verbenone could be responsible for the antibacterial properties of suico EO in this work. Additionally, the presence of eucalyptol may also have contributed to this antibacterial effect.

The EO of mint presented the lowest antibacterial activity against *S. scabiei*. Menthol has been described as a good to moderate antibacterial agent, while menthone was defined as less successful than menthol (Işcan *et al.*, 2002). Other

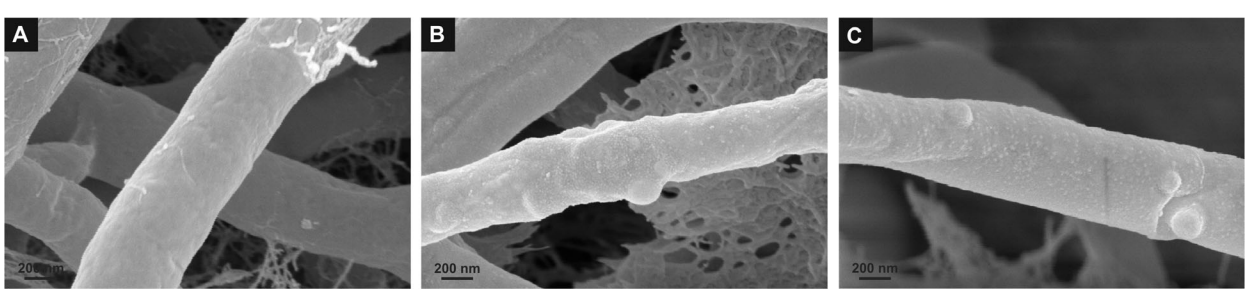

Fig. 1. Scanning electron microscopy microphotographs of *S. scabiei*^{A} vegetative hyphae exposed to different treatments: (A) control, (B) suico EO, (C) thyme EO.

compounds present in lower concentrations, such as eucalyptol and β-caryophyllene, could also be responsible for the antibacterial function of the EO of mint. To date, neomenthol, isomenthone, piperitenone and menthyl acetate have not been evaluated as antibacterial agents, and their contribution to the antibacterial effect is therefore unknown. In comparison with the others tested EO, mint EO was less effective.

All tested EO were effective against *S.* scabiei, with MIC $< 1\ g{\cdot}l^{-1}$, with thyme and suico EO being the most effective. Interestingly, the EO of oregano with a high TPC value, had low antibacterial properties in comparison with the other tested EO, while the EO of suico, with low TPC concentrations, is a good antimicrobial agent against *S. scabiei*, suggesting that the TPC content is not directly related to the antibacterial properties. Suico and thyme EO had highest bioactivity against *S. scabiei* but differed strongly in their chemical composition: thyme EO is rich in phenols, while suico EO has a ketone-dominated composition. Nevertheless, the SEM microphotographs showed a similar effect produced by EO of both species on the cell envelope of *S. scabiei*, suggesting a similar mechanism of action.

According to the obtained results, the tested EO are effective in control of *S. scabiei in vitro*. However, it would be important to develop an effective method that protects these EO from degradation conditions present under field conditions, such as heat, light and oxygen, and that deliver the EO gradually. The inclusion of the EO in a stable nanoemulsion or microcapsule is a promising solution.

CONCLUSION

Of the 31 bacterial isolates obtained, 12 were considered pathogenic and seven had the *txtAB* genes. Comparison of the *rpoB* gene showed the presence of different *Streptomyces* species in tuber lesions *versus* soil samples. One isolate (M7), obtained from infected potato tubers, was identified as *S. scabiei*. The EO of thyme and oregano were rich in phenols, while the EO of suico had a ketone-dominated composition. All EO showed antibacterial activity against *S. scabiei* with MIC $< 1\ g{\cdot}l^{-1}$. Thyme and suico EO were the most effective and had similar visual effects on the cell envelope of *S. scabiei*.

In summary, the tested EO seem to be promising tools for potato common scab control. Future research needs to confirm these findings through the application of the studied EO in field experiments. In addition, future experiments are needed to test possible phytotoxic effects of EO on potato plants, how they affect tuber sensory characteristics, and assess which is the best application method.

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