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Biopesticidal value of *Senecio glaucus* subsp. *coronopifolius* essential oil against pathogenic fungi, nematodes, and mites

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ABSTRACT

Essential oils are widely investigated as an alternative or complementary approach to broad-spectrum synthetic pesticides. In the present study, essential oil of *Senecio glaucus* subsp. *coronopifolius* was screened for its antifungal, nematicidal, acaricidal and repellent activities against the phytopathogens *Botrytis cinerea*, *Meloidogyne javanica* and *Tetranychus urticae* Koch under laboratory conditions. The volatile phase method (VF) and poisoned food method (PF) were adopted to test the antifungal activity of the essential oil. The oil gave 83% inhibition at 16 µL/ml using the PF method. VF method showed 86% inhibition of mycelial growth of *B. cinerea* at 0.8 µL/ml air. To check the nematicidal activity of the essential oil, two bioassays targeted the mortality of second stage juvenile (J2) and hatch inhibition of *M. javanica* eggs. 95% immobility of J2 and 92% inhibition of egg hatch were recorded at 16,000 ppm. The effect exerted by the oil was nematostatic. In the case of mites, a leaf dip bioassay revealed 100% mortality of *T. urticae* adults, and repellency of 24% after exposure to the oil at 80% concentration. Chemical profile of *S. glaucus* essential oil was obtained using GC–MS. Terpenic compounds, such as indicated α-pinene (26.2%), myrcene (11.4%), p-cymene (9.9%), β-pinene (7.7%), γ-muurolene (4%), deoxynivalenol (3.1%) and α-phellandrene (2.7%) were identified as the major components. The findings indicated that *S. glaucus* essential oil has the potential for management of these pathogens.
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1. Introduction

A significant escalation in global food production will be mandatory to feed the world's growing population by 2050 [1]. 20–40% of crop yields is lost to various pests and diseases. Pests for crop products include animal pests like insects, mites and nematodes; plant pathogens like fungi, bacteria and viruses, in addition to weeds [2]. All of which reduce crop production worldwide, resulting in losses reaching around $US100 billion annually [3]. So, controlling them is an essential economic and agricultural issue [4]. Farmers rely on the widespread use of synthetic pesticides to manage pests and diseases. However, their use is set to decline, as they are not always affordable, and may have harmful effects on plants, soil, beneficial organisms and human health. Additionally, their continued use leads to increasing pest resistance [4,5]. In view of this, efforts are being made worldwide to replace conventional pesticides through safer, environmentally friendlier and efficient alternatives, with a particular focus on biopesticides.

The use of natural products of plant origin has been proposed in recent years [6]. Plants essential oils (EOs) are optimal alternatives, since they are highly active and economically viable [7]. They are natural volatile substances produced by a variety of plants, which constitute a complex mixture of terpenoids and aromatic phenols.

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In the last decade, much effort has been devoted to screening plant EOs as potential tools for pest management [8]. Essential oils have been shown to possess antifungal, nematicidal and insecticidal activities [4,8–14].

The Asteraceae family is one of the main families of plants [15]. *Senecio* L. (Senecioneae Cass.) is the largest genus in this family. It includes over 1500 species distributed worldwide. Among them, 22 species are found in Morocco [16]. *Senecio* species have been used in folk medicine as anti-inflammatory, antiemetic, emmenagogue, vasodilatator, stomach pain reliever and for treatment of hepatic disorders, wounds, coughs, fever, colds, asthma, bronchitis, and eczema [17–21]. Essential oils isolated from *Senecio* species possess antibacterial [22–25], antifungal [26,27] and insect-repellent [17] activities. *Senecio glaucus* L. is an annual herb that belongs to this genus. It has two subspecies: subsp. *glaucus* and subsp. *coronopifolius*. The latter is a highly widespread species, with a natural range from south-west Asia, through North Africa in Southern Morocco, Tunisia and Egypt to the Canary Islands. It is found in agrestral and ruderal sandy fields, stony and rock slopes and river banks [28]. To the best of our knowledge, no studies have been published about the chemical composition and biological activities of EO from this species growing in Morocco.

The present study reports the chemical profile of EO isolated from Moroccan *Senecio glaucus* ssp. *coronopifolius*, and investigates its potential as biopesticide against plant pathogenic fungus (*Botrytis cinerea*), mite (*Tetranychus urticae*), and plant nematode (*Meloidogyne javanica*).

2. Materials and methods

2.1. Plant material and extraction of essential oil

Whole plants of *S. glaucus* ssp *coronopifolius* (stems, leaves, flowers and roots) were collected during flowering and ripening stages in April and May of 2017, from Massa locality in the South-West of Morocco. Essential oil was obtained using Clevenger-type apparatus through hydro-distillation for 4 h, which provides 0.08% yield.

2.2. Analysis of the chemical composition of essential oil

The chemical composition of the EO obtained from whole plants was identified and quantified by gas chromatography coupled with mass spectrometry (GC/MS) [29]. The GC was carried out using a QP 2010 Plus gas chromatography apparatus (Shimadzu, Tokyo, JP) equipped with an automatic injector (AOC-5000, Belle-fonte, USA), and an Rtx-5MS capillary column (30 m × 0.25 mm ID × df 0.25 µm, 5% diphenyl/95% dimethyl polysiloxane) (Restek, Bellefonte, USA). Helium was used as a carrier gas (100, 2 kPa). About 1 µL of EO diluted in dichloromethane (Merck, Darm-stadt, GE) at concentration of 1% (10 mg/mL) was injected using 1:10 split ratio. The injector temperature was 250 °C and the temperature at the interface between the column and the ion source was set to 250 °C. The oven temperature was regulated to obtain a linear ramp from 60 to 246 °C (3 °C/min) [30]. The mass scan ranged from 50 to 465 *m*/*z*. and the ionization energy in the source was 70 eV. The Van der Dool and Kratz Index were calculated as an additional tool for compounds identification [30]. For this, a mixture of hydrocarbons ($C_{10}H_{22}$-$C_{40}H_{82}$) (Fluka, St. Louis, USA) was injected under the same conditions.

The spectrum processing and identification was performed using the GC–MS Solutions V2.5 software, which is coupled with the NIST11 and NIST11s database libraries containing recorded spectra.

2.3. Test organisms

B. cinerea was isolated directly from rotten beans (*Phaseolus vulgaris*) infected by the fungus. The fungus was maintained on PDA at 4 °C. Eggs of the nematode *M. javanica* were extracted from infected bean roots. Second-stage juveniles (J2) were obtained from the water suspension of infected roots containing hatched eggs, and stored at 4 °C until used. The two-spotted spider mite was obtained from the SAOAS Group Company, natural enemy production unit, Morocco.

2.4. Antifungal activity

2.4.1. Poisoned food technique

The poisoned food technique (PF) was used according to Rhayour et al. [31] method, with modifications. The EO was dispersed as an emulsion in sterile PDA using Tween 80 0.2%. The concentrations tested ranged from 0.25 to 16 µL/mL. Controls consisted of sterile PDA mixed with 0.02% Tween 80. The tested fungi was inoculated using a 6 mm mycelial plug from a 7-day-old culture. Three replicate plates were inoculated for each treatment; plates were incubated for 7 days at 25 ± 2 °C.

2.4.2. Volatile phase technique

Volatile activity assay (VA) was performed following the method of Soylu et al. [32], with some modifications. Petri dishes (9 cm) were filled with 20 mL of PDA medium (80 mL air space) and then seeded with a mycelial disk (6 mm diameter) cut from the periphery of a 7-day-old culture of the fungi. Petri dishes were inverted, and sterile Whatman No. 1 filter paper discs (10 mm diameter) impregnated with different volumes of EO were deposited on the inverted lid to obtain final concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 µL/mL air, and incubated for 7 days at 25 ± 2 °C. Controls of sterilized filter paper discs impregnated with 20 µL/disc of distilled water were used. Three replicate plates were inoculated for each treatment, and plates were incubated for 7 days at 25 ± 2 °C.

The fungitoxicity of the EO is expressed as percent inhibition of mycelial growth (I%) and evaluated according to the formula of Pandey et al. [33] (Eq. (1)).

$$I(\%) = ((Di - Dt))/Di \times 100 \quad (1)$$

where Di is mycelial growth diameter in control, Dt is mycelial growth diameter in treated petri plates.

2.5. Nematicidal activity

2.5.1. Hatching inhibition of eggs and nematicidal activity on second stage juveniles

The experiment was carried out in 96-well microplates following a modified method of Sosa et al. [34]. Approximately 50 eggs or 50 freshly hatched J2s of *M. javanica* were placed in each well prior to adding the EO. The oil was diluted with water containing 0.2% Tween 20 v/v, as the surfactant to obtain the desired oil concentrations. The tested concentrations were 800, 8000, 10,000, 12,000, 14,000 and 16,000 ppm. Distilled water and a 0.2% Tween 20 solution were included as controls. The microplate was maintained at 25 °C. Hatching percentage was recorded for 10 days, and percentage of immobilized J2 was recorded for 3 days using an inverted microscope. Lack of mobility was taken as evidence of the effect of the tested solutions. Each treatment had four replicates. Data of mortality and hatch inhibition (Crd) was corrected according to the formula of Abbott [35] (Eq. (2)).

$$Crd(\%) = ((Pmtr - Pmc))/((100 - Pmc)) \times 100 \quad (2)$$

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where Pmtr is egg hatching percentage or dead J2 percentage in treated wells, Pmc is egg hatching percentage or dead J2 percentage in the control.

2.5.2. Demonstration of the nematostatic effect

Second stage juveniles of *M. javanica* (approximately 50) were exposed to the action of the EO of *S. glaucus* at the highest concentration of 16,000 ppm. The suspension of the treated J2 was passed through a 50 μm sieve while being washed with distilled water to eliminate the effect of the EO. Larvae suspended in distilled water were recovered in petri plates. The vitality of immobile nematodes was evaluated using Meldola Blue staining technique as per Ogiga and Estey [36]. A few drops of Meldola's blue dye were added to each plate to distinguish the dead J2 from the paralyzed ones. Dead larvae were stained purple-blue while paralyzed larvae remain uncolored. Four replicates were performed. Two controls were used: the first consisted of J2 larvae suspended in distilled water and the second were J2 treated with heat (65 °C for 24 h). After one hour, the dead and the paralyzed larvae were counted under an inverted microscope. The percentage of J2 resuming their mobility was determined after 24, 48 and 72 h to study the reversibility of paralysis of J2.

Three parameters were determined: the larval mortality rate (Mr), the paralysis rate (Pr) and the percentage of J2 having resumed their mobility (Rm). The three parameters were calculated and corrected against the control according to the formula of Abbott [35] (Eqs. (3)–(5)).

$$Mr = (\text{number of dead J2})/(\text{total number of J2}) \times 100 \quad (3)$$

$$Pr = (\text{number of paralyzed J2})/(\text{total number of J2}) \times 100 \quad (4)$$

$$Rm = (\text{number of J2 having resumed mobility})/(\text{total number of J2}) \times 100 \quad (5)$$

2.6. Insecticidal activity

2.6.1. Acaricidal activitym

A modified leaf dip bioassay was used to test acaricidal activity, according to the method of Ebadollahi et al. [37]. Five concentrations including 0.1, 1, 10, 50 and 80% of *S. glaucus* EO were prepared with Tween-80 at 0.02% as an emulsifier. The bioassay was conducted in petri dishes (9 cm diameter). Fresh leaves of bean were collected from unsprayed plants growing in a greenhouse (INRA experimental farm, Agadir, Morocco). Leaves were immersed in prepared concentrations for 10 s. After drying at room temperature, each leaf was individually placed in the bottom of a petri dish atop a 9 cm diameter disc of Whatman paper wetted with distilled water. Ten adult mites were introduced into each petri dish, put on top of the leaf and then covered. In control groups, mites were held on leaves immersed in distilled water mixed with the adjuvant (Tween-80). Three replicates for each treatment were used, and mortality rate was assessed at 24, 48, 72 h, 6, 7 and 8 days after treatment. The bioassay was replicated three times, and mortality rates (CrrM) were corrected using the formula of Abbott [35] (Eq. (6)).

$$CrrM[\%] = (dmn - dmnc)/(mtn - dmnc) \times 100 \quad (6)$$

where dmn is the number of dead mites in treatments, dmnc is the number of dead mites in control, mtn is the total mite number.

2.6.2. Repellent activity

A modified choice test was used for a repellent activity assay according to the method of Pascual-Villalobos and Robledo [38]. Two boxes were used, one contained the treated leaves and the other represented the control (untreated leaves). The two boxes were connected with an 8 cm long translucent hose (1 cm in diameter) that was pierced in the middle, to allow the introduction of *T. urticae* adults. Test solutions were prepared by diluting the EO in Tween-80 0.02%. The concentrations tested were 0.1, 1, 10, 50 and 80% of EO. Fresh bean leaves were immersed for 10 s in either an EO solution or distilled water, air dried for 5 min, and then placed in the corresponding box. Fifteen *T. urticae* adults were transferred gently through the hole made in the center of the linked-hose, after which the hole was sealed. This structure allowed mites to move freely to both boxes (control or treated box). The repellent effect was assessed at 24, 48 and 72 h after release, and three replicates were made for each treatment. A repellency index (RI) was determined for each treatment at every period of time (24, 48 and 72 h) using the formula of Pascual-Villalobos and Robledo [38] (Eq. (7)).

$$RI = (C - T)/(C + T) \times 100 \quad (7)$$

where C is the number of mites in the control box, and T is the number of mites in the treated box.

2.7. Statistical analysis

The data collected were subjected to one-way analysis of variance ANOVA at $p \leq 0.01$ using STATISTICA 6. Newman-Keuls test was used to compare resulting means. Probit regressions were used to calculate median inhibitory concentration IC_{50}, median lethal concentration LC_{50} and median lethal dose LD_{50} values at 95% confidence limit, using the Polo-PC software [39].

3. Results

3.1. Essential oil chemical composition

The results of chemical analysis of *S. glaucus* EO by GC–MS are shown in Table 1. Eighty five compounds were identified, representing 94.3% of the total constituents. 86.5% of the total identified compounds were terpenic ones. Monoterpene hydrocarbons had the major percentage (59.2%), followed by sesquiterpenoids (11.5%). The lowest percentage was attributed to monoterpenoids (6.9%). The major compounds detected were α-pinene (26.2%), myrcene (11.4%), p-cymene (9.9%), β-pinene (7.7%), γ-muurolene (4%), deoxynivalenol (3.1%) and α-phellandrene (2.7%).

3.2. Antifungal activity

The effects of *S. glaucus* EO on mycelial growth of *B. cinerea* are presented in Fig. 1(a) and (b). Using the PF technique, percent inhibition increased significantly with concentration and reached its maximum value of 83% at 16 μL/mL. Similarly, for the VA technique, inhibition percentage increased significantly with increasing quantity of EO. The highest inhibition of 86% was obtained with the highest concentration tested (0.8 μL/mL air). IC_{50} values for PF and VA techniques were 8759 μL/mL and 0.77 μL/mL air respectively.

3.3. Nematicidal activity

3.3.1. Hatching inhibition of eggs and nematicidal activity on second stage juveniles

Hatch inhibition data of *M. javanica* eggs is presented in Table 2. The hatch inhibition rate increased with oil concentration. At a concentration of 16,000 ppm, the highest egg hatch inhibition rate detected was 92.96%. LC_{50} value was 9956 ppm. The nematicidal effect of the EO against second stage juvenile (J2) increased with concentration and incubation time (Fig. 2). After three days of incu-

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Table 1
Chemical composition of essential oil isolated from *S. glaucus* subsp. *coronopifolius.*

Compound	RI_E	Content%	Molecule formula
α-Pinene	948	26.2	$C_{10}H_{16}$
Myrcene	958	11.4	$C_{10}H_{16}$
p-Cymene	1042	9.9	$C_{10}H_{14}$
β-Pinene	943	7.7	$C_{10}H_{16}$
γ-Muurolene	1435	4.0	$C_{15}H_{24}$
Deoxynivalenol	2385	3.1	$C_{15}H_{20}O_6$
α-Phellandrene	969	2.7	$C_{10}H_{16}$
Eremophila-1(10),11-diene	1474	1.8	$C_{15}H_{22}O$
Anthracene, 1,2,3,4,5,6,7,8-octahydro-9,10-dimethyl-	1879	1.4	$C_{16}H_{22}$
Terpinen-4-ol	1137	1.3	$C_{10}H_{18}O$
Ledene oxide-(II)	1293	1.3	$C_{15}H_{24}O$
Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-	1599	1.2	$C_{15}H_{24}O$
Spathulenol	1536	1.0	$C_{15}H_{24}O$
1-Octen-3-yl-acetate	1109	1.0	$C_{10}H_{18}O_2$
Cyclopropane, 1-methyl-2-pentyl-	880	1.0	C_9H_{18}
2-Butenoic acid, 2-methyl-, 1a,2,4,4a,5,9-hexahydro-4,4a,6-trimethyl-3H-oxireno[8,8a]naphtho[2,3-b]furan-5-yl ester	2109	0.9	$C_{20}H_{26}O_4$
Caryophyllene oxide	1507	0.9	$C_{15}H_{24}O$
Curcumene	1524	0.9	$C_{15}H_{22}$
α-Campholenal	1155	0.8	$C_{10}H_{16}O$
Cyclohexene, 3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl-	1471	0.8	$C_{12}H_{20}O_3$
Hexahydrofarnesyl acetone	1754	0.7	$C_{18}H_{36}O$
Aromadendrene oxide-(2)	1462	0.6	$C_{15}H_{24}O$
Naphthalene, 1,2,3,4,4a,5,8,8a-octahydro-4a-methyl-, trans-	1157	0.6	$C_{11}H_{18}$
Caryophyllene	1494	0.6	$C_{15}H_{24}$
Cadina-3,9-diene	1440	0.5	$C_{15}H_{24}$
Perillene	1125	0.5	$C_{10}H_{14}O$
2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	1502	0.5	$C_{15}H_{24}$
Isoaromadendrene epoxide	1281	0.5	$C_{15}H_{24}O$
γ-Terpinene	998	0.5	$C_{10}H_{16}$
(1*R*)-(−)-Myrtenal	1136	0.5	$C_{10}H_{14}O$
Isopinocarveol	1131	0.4	$C_{10}H_{16}O$
α-Terpineol	1143	0.4	$C_{10}H_{18}O$
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1alpha,2beta.,4beta)]-	1398	0.4	$C_{15}H_{24}$
α-Guaiene	1490	0.4	$C_{15}H_{24}$
cis-Verbenol	1136	0.4	$C_{10}H_{16}O$
Carvacrol	1262	0.4	$C_{10}H_{14}O$
Terpinolene	1052	0.3	$C_{10}H_{16}$
4,5-di-epi-Aristolochene	1474	0.3	$C_{15}H_{24}$
Cumaldehyde	1230	0.3	$C_{10}H_{12}O$
Phellandral	1175	0.3	$C_{10}H_{16}O$
β-Vatirenene	1489	0.3	$C_{15}H_{22}$
β-d-Mannofuranoside, farnesyl-	3014	0.3	$C_7H_{14}O_6$
p-Mentha-6,8-dien-2-ol, cis-	1206	0.2	$C_{12}H_{18}O_2$
(−)-β-Bourbonene	1339	0.2	$C_{15}H_{24}$
Alloaromadendrene	1386	0.2	$C_{15}H_{24}$
Cubenol	1580	0.2	$C_{15}H_{26}O$
Tau-muurolol	1580	0.2	$C_{15}H_{26}O$
α-Terpinene	998	0.2	$C_{10}H_{16}$
Geranyl linalool	2046	0.2	$C_{20}H_{34}O$
Eudesma-5,11(13)-dien-8,12-olide	1801	0.2	$C_{15}H_{20}O_2$
1-Isopropyl-4-methylenebicyclo[3.1.0]hex-2-ene	879	0.2	$C_{10}H_{14}$
Pinocarvone	1114	0.2	$C_{10}H_{14}O$
2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	1745	0.2	$C_{15}H_{24}O$
2-(1,4,4-Trimethyl-cyclohex-2-enyl)-ethanol	1313	0.2	$C_{11}H_{20}O$
4-Piperidyl cyclopentylphenylglycolate	2498	0.2	$C_{18}H_{25}NO_3$
Phytol	2045	0.2	$C_{20}H_{40}O$
Heneicosane	2109	0.2	$C_{21}H_{44}$
4(10)-Thujen-3-ol, acetate	1224	0.1	$C_{12}H_{18}O_2$
Ethanol, 1-(1-cyclohexenyl)-	1053	0.1	$C_8H_{14}O$
Di-epi-α-Cedrene	1403	0.1	$C_{15}H_{24}$
α-Humulene	1579	0.1	$C_{15}H_{24}$
Aromadendrene oxide-(1)	1462	0.1	$C_{15}H_{24}O$
Podocarp-13-en-12-ol	1861	0.1	$C_{17}H_{28}O$
α-Thujene	902	0.1	$C_{10}H_{16}$
Longipinocarveol, trans-	1599	0.1	$C_{15}H_{24}O$
6.β.Bicyclo[4.3.0]nonane, 5.β.-iodomethyl-1.β.-isopropenyl-4α,5α-dimethyl-	1831	0.1	$C_{15}H_{25}$
Seychellene	1275	0.1	$C_{15}H_{24}$
p-Mentha-1(7),8-dien-2-ol	1201	0.1	$C_{10}H_{16}O$
Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-	1599	0.1	$C_{15}H_{24}O$
Phytol, acetate	2168	0.1	$C_{22}H_{42}O_2$
1-Methylverbenol	1209	0.1	$C_{10}H_{16}O$
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	1690	0.1	$C_{15}H_{24}O$
Kauran-18-al, 17-(acetyloxy)-, (4.β.)-	2338	0.1	$C_{22}H_{34}O_3$
(*E*,*E*)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	1922	0.1	$C_{20}H_{32}$

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Table 1 (*continued*)

Compound	RI_E	Content%	Molecule formula
Bicyclo[10.1.0]tridec-1-ene	1472	0.1	$C_{13}H_{22}$
Camphene	943	0.1	$C_{10}H_{16}$
3a-Methyl-3a,4,5,6,7,8-hexahydro-2(3H)-azulenone	1357	0.1	$C_{11}H_{16}O$
p-Cymen-7-ol	1284	0.1	$C_{10}H_{14}O$
Guaia-1(10),11-diene	1490	0.1	$C_{15}H_{24}$
2-Butenoic acid, 2-methyl-, 4,4a,5,6,7,9-hexahydro-3,4a,5-trimethylnaphtho[2,3-b]furan-4-yl ester, [4S-[4.α.(Z),4a.α.,5.α.]]-	2219	0.1	$C_{20}H_{28}O_4$
(+)-Sativene	1339	0.1	$C_{15}H_{24}$
Cycloisolongifolene, 8,9-dehydro-9-formyl-	1457	0.1	$C_{16}H_{22}O$
2-Undecanone, 6,10-dimethyl-	1321	0.1	$C_{13}H_{26}O$
Decane	1015	0.1	$C_{10}H_{22}$
2-Caren-10-al	1136	0.1	$C_{10}H_{14}O$
Monoterpene hydrocarbons		59.2	
Oxygenated monoterpenes (monoterpenoids)		6.9	
Sesquiterpene hydrocarbons		8.9	
Oxygenated sesquiterpenes (sesquiterpenoids)		11.5	
Others		7.8	
Total		94.3	

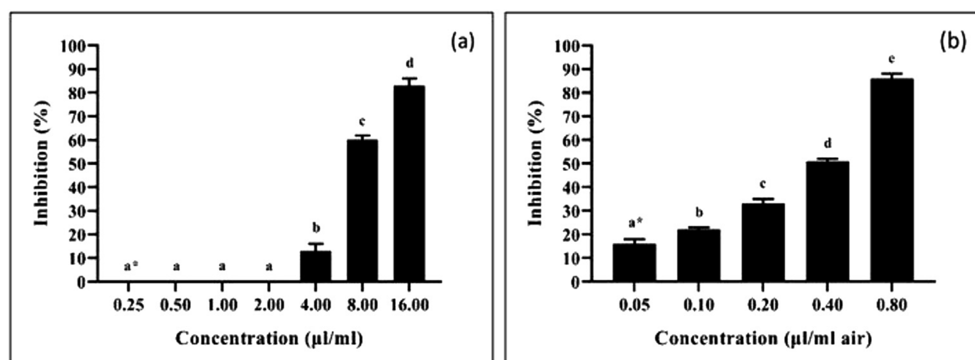

Fig. 1. Inhibition percentages of essential oil of *S. glaucus* subsp. *coronopifolius* against *Botrytis cinerea* at different concentrations using poisoned food (a) and volatile phase methods (b) *Values assigned the same letter do not differ significantly, according Newman-Keuls test ($p \leq 0.01$).

bation, the concentration of 16,000 ppm produced the highest immobility rate with 95% immobility, and an LC_{50} value of 9973 ppm was recorded.

3.3.2. Demonstration of the nematostatic effect

Immobile J2 exposed to the effects of the oil at 16,000 ppm were not stained by the blue Meldola similarly to the heat-treated J2 controls, which were stained dark purple. The percentage of dead J2 was very small compared to the paralyzed fraction. It reached 11.5% after three days of exposer. The rate of paralysis was significant, and tended to decrease slightly during the three days of exposure with the increase in mortality rate. On the third day, it reached 88.5% (Fig. 3). The oil has considerable nematostatic potential. Regarding the reversibility of *M. javanica* J2 paralysis, the oil was able to maintain the paralysis of immobile J2 for three days of incubation. No paralyzed J2 has recovered its mobility.

3.4. Insecticidal activity

3.4.1. Acaricidal activity

The acaricidal activity of the EO against adults of *T. urticae* is presented in Table 3. *S. glaucus* EO provided significant mortality to *T. urticae* adults 24, 48 and 72 h after treatment. The earliest deaths occurred within 24 h after application. Activity of the oil was enhanced with increasing amount of doses. At the highest concentration, *T. urticae* mortality rate exceeded 50% 72 h after application. 192 h after application of the oil, the mortality rate ranged from 32 to 100% at concentrations 50–80%. Probit analysis indicated an LD_{50} of 5843 ppm.

3.4.2. Repellent activity

S. glaucus EO repelled *T. urticae* adults after 24, 48 and 72 h of exposure (Fig. 4). The level of repellency observed for each treatment did not differ much after 24, 48 or 72 h. The EO had an attractive effect at concentrations 0.1, 1 and 10% with the RI ranged from −68 to −3. After 72 h, low repellent effect was seen on *T. urticae* adults at the highest concentration tested with an RI of 24%.

4. Discussion

The major constituents of *S. glaucus* EO mainly belonged to four chemical groups: monoterpene hydrocarbons (i.e. α-pinene, myrcene, p-cymene, β-pinene and α-phellandrene), sesquiterpene hydrocarbons (i.e. γ-muurolene), oxygenated sesquiterpenes (i.e. deoxynivalenol, eremophila-1(10),11-diene and ledene oxide-(II)) and oxygenated monoterpenes (i.e. terpinen-4-ol and 1-octen-3-yl-acetate). Comparing this chemical profile with that described in an earlier study by De Pooter et al. [40] on oil of Egyptian *S. glaucus* revealed some differences. In that study, myrcene was the main compound, detected in a higher amount (24% vs. 11.49%), followed by dehydrofukinone (21%), which was not detected in our oil, and p-cymene identified at the same amount as in our oil (9.9%). Interestingly, α-pinene the chief component of our oil was present in Egyptian oil at a low amount (2.6% vs. 26.23%). Differences between oils may be related to several factors, such as

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Table 2
Effect of *S. glaucus* subsp. *coronopifolius* essential oil on hatch inhibition of eggs of *Meloidogyne javanica.*

Concentration (ppm)	Days after incubation									
	1	2	3	4	5	6	7	8	9	10
800	11.54 ± 3.77 a, b*	18.75 ± 2.50 a,b, c	13.46 ± 3.62 a,b	11.67 ± 2.33 a,b	16.67 ± 2.33 a,b, c	9.38 ± 2.81 a	9.26 ± 1.63 a	11.40 ± 1.14 a,b	19,70 ± 1.03 a,b,c	19,72 ± 2.23 a,b,c
8000	23.08 ± 2.69 a,b, c	28.13 ± 3.38 a,b, c,d	48.08 ± 4.46 a,b,c, d,e,f,g,h	36.67 ± 4.00 a,b, c,d,e,f	36.11 ± 2.94 a,b, c,d,e,f	45.83 ± 2.33 a,b,c, d,e,f,g	49.07 ± 3.13 a,b,c, d,e,f,g,h,i	46.49 ± 5.11 a,b,c, d,e,f,g	46.21 ± 5.11 a,b,c, d,e,f,g	45.07 ± 5.42 a,b,c, d,e,f,g
10,000	42.31 ± 6.85 a,b, c,d,e,f,g	21.88 ± 2.94 a,b, c	51.92 ± 1.73 a,b,c, d,e,f,g,h,i,j	38.33 ± 5.00 a,b, c,d,e,f	44.44 ± 4.22 a,b, c,d,e,f,g	55.21 ± 5.79 b,c,d, e,f,g,h,i,j,k	59.26 ± 5.52 c,d,e, f,g,h,i,j,k	55.26 ± 5.18 b,c,d, e,f,g,h,i,j,k	53.79 ± 4.91 a,b,c, d,e,f,g,h,i,j	50.00 ± 4.20 a,b,c, d,e,f,g,h,i
12,000	34.62 ± 6.23 a,b, c,d,e	46.88 ± 5.63 a,b, c,d,e,f,g	67.31 ± 3.62 d,e,f,g, h,i,j,k	70.00 ± 3.00 d,e, f,g,h,i,j,k	75.00 ± 2.33 e,f, g,h,i,j,k	76.04 ± 2.38 e,f,g,h, i,j,k	72.22 ± 4.26 d,e,f, g,h,i,j,k	69.30 ± 2.58 d,e,f,g, h,i,j,k	68.18 ± 0.27 d,e,f,g, h,i,j,k	66.90 ± 0.46 d,e,f, g,h,i,j,k
14,000	76.92 ± 5.23 e,f, g,h,i,j,k	75.00 ± 5.00 e,f, g,h,i,j,k	84.62 ± 4.38 g,h,i,j, k	81.67 ± 2.67 f,g, h,i,j,k	81.94 ± 3.19 f,g, h,i,j,k	86.46 ± 3.90 g,h,i,j, k	87.96 ± 2.80 g,h,i, j,k	86.84 ± 3.65 g,h,i,j, k	81.82 ± 1.58 f,g,h,i, j,k	78.87 ± 1.75 e,f,g, h,i,j,k
16,000	100.00 ± 0.00 k	100.00 ± 0.00 k	96.15 ± 1.77 j,k	93.33 ± 2.00 h,i,j, k	94.44 ± 1.33 i,j,k	95.83 ± 1.25 j,k	96.30 ± 1.56 j,k	94.74 ± 2.89 i,j,k	93.18 ± 2.82 h,i,j,k	92.96 ± 2.04 h,i,j,k

* The rates followed by the same letters are not statistically different at $p \leq 0.01$ according to the Newman-Keuls test.

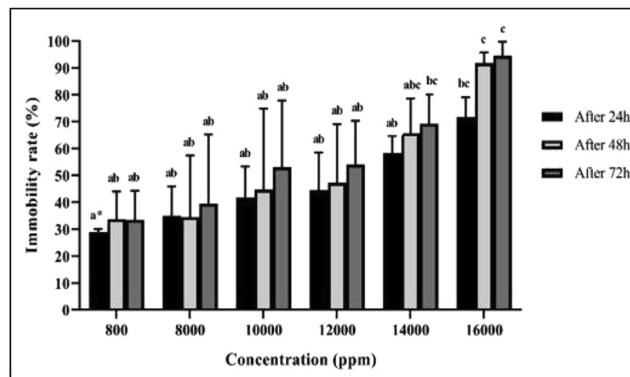

Fig. 2. Effect of *S. glaucus* subsp. *coronopifolius* essential oil on immobility of second stage juveniles of *Meloidogyne javanica*. *Values followed by the same letter do not differ significantly according to the test of Newman- keuls ($p \leq 0.01$).

geographical location, environmental conditions, collect season and nutritional status of plants [41,42].

Essential oils and their major constituents, monoterpenes, are a highly promising class of natural products that can be used as safer pest and disease control agents [43,84]. In this study, *S. glaucus* EO exhibited antifungal, nematostatic, acaricidal and repellent activities. Antifungal activity has been reported with other species of the genus *Senecio*. Essential oil of *Senecio amplexicaulis* showed high antifungal activity against *Sclerotium rolfsii, Macrophomina phaseolina, Rhizoctonia solani, Pythium debaryanum* and *Fusarium oxysporum* [27]. Similarly, EOs of *Senecio nutans* and *Senecio viridis* have been shown actives against *Fusarium* spp. [26]. At low concentrations, *S. glaucus* oil provided weak inhibition of mycelial growth of *B. cinerea*. This comes in agreement with findings of Wilson [85], who reported weak antifungal activity of *Senecio obovatus* plant extract against the grey mould agent at 10% concentration. *S. glaucus* oil gave different results of inhibition with different methods used. It provided better inhibition using the volatile phase method compared to the poisoned food method. This may be attributed to the hydrophobic nature of EOs, which results in low solubility of the oils in the agar medium that contains water [44]. These results are supported by previous studies [32,44,45]. Volatile compounds of EOs, primarily monoterpene and sesquiterpene hydrocarbons, are accountable for their biological activity [46]. Sesquiterpenes isolated from members of the Asteraceae, possess a wide spectrum of biological activities, including antifungal activity against *B. cinerea* [47]. As

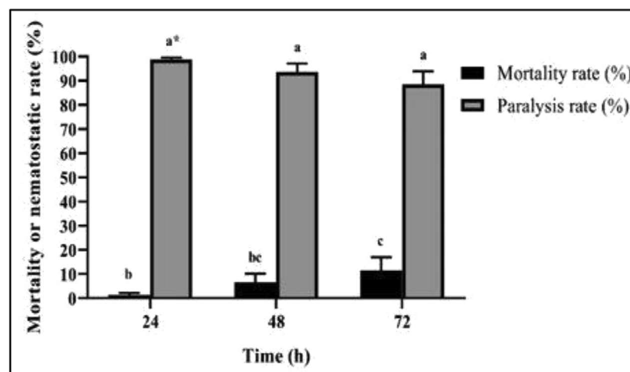

Fig. 3. Nematicidal and nematostatic effects of *S. glaucus* subsp. *coronopifolius* essential oil on second stage juveniles of *Meloidogyne javanica*. *Values followed by the same letter do not differ significantly according to the test of Newman- keuls ($p \leq 0.01$).

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Table 3
Effects of *S. glaucus* subsp. *coronopifolius* essential oil on *Tetranychus urticae* adults mortality rates.

Concentration (%)	Hours after application					
	24	48	72	144	168	192
0.1	2.4 ± 7.0 ab*	7.5 ± 8.9 abcd	6.2 ± 7.2 abc	2.6 ± 5.3 bcdef	8.8 ± 5.4 abcde	0.0 ± 6.5 a
1	7.6 ± 4.6 abcd	12.7 ± 5.6 bcde	12.3 ± 7.0 bcdef	12.7 ± 4.6 bcde	10.2 ± 6.0 abcde	3.2 ± 6.5 ab
10	11.8 ± 4.9 bcde	17.0 ± 5.6 cdefg	20.3 ± 5.6 efg	23.9 ± 5.3 gh	23.5 ± 8.8 fgh	18.0 ± 9.3 defg
50	19.4 ± 5.1 efg	25.6 ± 6.3 ghi	32.7 ± 7.1 hij	36.5 ± 8.6 j	35.2 ± 8.8 ij	32.8 ± 10.7 hij
80	27.8 ± 5.9 ghij	36.7 ± 7.1 j	52.2 ± 7.0 k	78.8 ± 9.4 l	89.7 ± 7.5 m	100 ± 0 n

* The rates followed by the same letters are not statistically different at $p \leq 0.01$ according to the Newman-Keuls test.

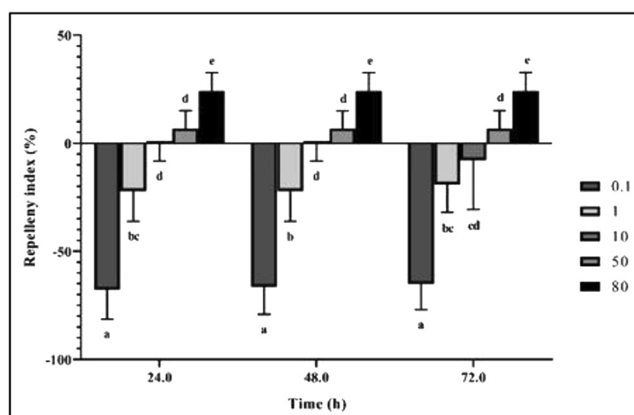

Fig. 4. Repulsive effect of *S. glaucus* subsp. *coronopifolius* essential oil on *Tetranychus urticae* adults. The repellency index (%) followed by the same letters do not differ at $p \leq 0.01$ according to the Newman-Keuls test.

well, major components of *S. glaucus* oil α-pinene, myrcene, p-cymene and β-pinene, have been reported as antifungal against *B. cinerea* and other fungi [43,48–54].

In relation to acaricidal and repellent activities against *T. urticae*, earlier reports showed the susceptibility of the two spotted spider mite to EOs derived from species belonging to the Asteraceae family. Essential oils of *Chamomilla recutita* and *Achillea millifolium* L. exhibited high mortality on female adults of *T. urticae* with LC_{50} values of 0.65 and 1.20% respectively. Similarly, *Artemisia absinthium*, *Tanacetum vulgare, Santolina africana* and *Hertia cheirifolia* EOs produced LC_{50} values of 0.130 mg/cm^2, 0.054 mg/cm^2, 2.35 and 3.43 ppm respectively on adults of *T. urticae* [37,55–58]. *S. glaucus* EO caused less toxicity to *T. urticae* in comparison with other oils from Asteraceae family, and weak repellency against adult mites. To the best of our knowledge, no oils from other *Senecio* species have been tested on *T. urticae*. The differences of toxicity between plant species are probably related to variances in chemical composition and proportion of the main constituents [59]. Furthermore, the choice of extraction technique affects oil composition qualitatively and quantitatively, which translates to bioactivity of the oil. Chiasson et al. [56] found that EOs obtained with direct steam distillation had higher toxicity on mites than the same oils extracted with water distillation or microwave assisted process. Therefore, several extraction techniques available need to be evaluated in order to enhance the effectiveness of the oil. The choice of the bioassay method also influences acaricidal activity, as some EOs were reported to be more toxic when applied as fumigants instead of being in direct contact with *T. urticae* [60]. Acaricidal effects of the oil against *T. urticae* may be attributed to its main monoterpenoidal compounds, as some of the major compounds of the oil such as α-pinene, β-pinene and p-cymene were demonstrated to display toxicity against this pest [7,61–64]. Nonetheless, their interaction with other minor constituents cannot be fully disregarded.

Potent antinematode potential was observed, with a significant reduction of egg hatch and J2 survival of the nematode, though it required high concentrations in oil. These results are comparable to findings of previous studies. Essential oils of *Achillea fragrantissima, Artemisia arboresses* and *Artemisia dracunculus* (Asteraceae) produced 28, 38 and 31% immobility of *M. javanica* J2 respectively at 1000 ppm [12]. Whereas, *S. glaucus* oil produced 34% immobility of J2 at 800 ppm. From *Senecio* genus, aqueous extract of *Senecio brasiliensis* produced 45% egg hatch inhibition and 40% immobility of *M. javanica* juveniles [59]. To the best of our knowledge, this is the first study on nematicidal activity on oils derived from species of the genus *Senecio*. Nematicidal bioactivity of plant oils has been related to their specific chemical profile [12]. Major recognized compounds in *S. glaucus* oil have been documented. Myrcene, α and β-pinene reduced hatching and J2 mobility of *M. incognita* [65,66], and p-cymene possessed nematicidal activity against juveniles of *M. incognita* [67].

Numerous studies have been done to investigate nematicidal potential of EOs against root knot nematodes. Nevertheless, these studies only report a lethal effect of the substances tested, as they associate immobility of nematodes with their death. Thus, a distinction between a lethal effect and a nematostatic effect of substances is vital. In this work, we showed that *S. glaucus* EO had a strong nematostatic effect on second stage juveniles of *M. javanica*, as it maintained the paralysis of immobile J2 three days after coloring with blue Meldola. The oil also exhibited a slightly lethal effect with 9.5% of J2 found dead. Previous studies reported the nematostatic effect of plant derived substances using Meldola Blue staining method, mainly with plant extracts [68–72]. To the best of our knowledge, this study is the first to report a nematostatic effect of plant EO using the Meldola Blue staining method.

Mechanisms of the biological activities of the EO are yet to be clearly elucidated. *S. glaucus* oil is made of a variety of diverse molecules, hence; antifungal activity may result from a combination of several modes of action, involving different target sites. It has been reported that monoterpenes act on cellular membranes. Owing to their hydrophobic properties, they penetrate the fungal membrane and induce physicochemical changes in the properties of the cell membrane and cellular organelles, resulting in membrane disruption and inhibition of respiration [73,74]. It was also stated that monoterpenes inactivate and/or inhibit the synthesis of intracellular and extracellular enzymes [75]. Major compounds of the oil α and β-pinene are able to destroy cellular integrity, and thereby, inhibit respiration and ion transport processes [76,77]. Furthermore, they inhibit microbial phospholipase and esterase activities [78]. Another major compound of *S. glaucus* oil p-cymene has been reported for contributing to antifungal activity. Being highly hydrophobic, it is easily incorporated into the lipid bilayer of the cytoplasmic membrane where it creates swelling to facilitate the penetration of other bioactive components [79].

On nematodes, the nematicidal action of the EO could be attributed to its capability of producing more intracellular reactive oxygen species (ROS) in nematodes, which results in severe oxidative damages leading to apoptosis and necrosis in nematodes [80,81].

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Other possible modes of action include the disruption and change of the permeability of nematode cell membranes [12], and interruption of the nematode nervous system by inhibiting acetylcholinesterase activity, with acetylcholine serving as a neurotransmitter in nematodes [82]. On the two-spotted spider mite, possible mechanisms are related to inhibition of acetylcholinesterase activity, interference with gama-aminobutyric acid receptor and transaminase activities [61,83]. Nonetheless, further investigation are required to explore these mechanisms at a deeper level.

5. Conclusion

The present study supports the biopesticidal action of plant derived EOs. It demonstrated that EO isolated from *S. glaucus* has potential as antifungal, antinematode, acaricidal and repellent agent, and may be suggested as a component of sustainable management of the pathogens here mentioned. Perspectives of this study would be (i) assessment of the effect of the oil in combination with other natural substances, (ii) study of the field effectiveness of the EO, in addition to (iii) testing its ability against other economical diseases and insect pests.

CRediT authorship contribution statement

Khadija Basaid: Investigation, Writing - original draft. **El Hassan Mayad:** Conceptualization, Methodology, Writing - review & editing. **Rachid Bouharroud:** Conceptualization, Methodology, Formal analysis, Writing - review & editing. **James Nicholas Furze:** Writing - review & editing. **Hinde Benjlil:** Writing - review & editing. **Alessandra Lopes de Oliveira:** Formal analysis. **Bouchra Chebli:** Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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