EFFECT OF TEMPERATURE, pH AND ESSENTIAL OILS ON THE MYCELIAL GROWTH OF *RHIZOCTONIA SOLANI* Kühn (CANTHARELLALES: CERATOBASIDIACEAE) ISOLATES

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ABSTRACT

This research aimed to study diversity among a collection of *Rhizoctonia solani* strains, isolated from many crops in Morocco, based on radial mycelial growth on potato dextrose agar (PDA) at different pH and temperature levels. The *in vitro* antifungal efficacy of essential oils (EOs) derived from aromatic and medicinal plants of *Thymus vulgaris, Origanum compactum, Rosmarinus officinalis, Eucalyptus* sp., *Salvia sp., Cistus ladanifer* and *Lavandula stoechas* was also evaluated by measuring the mycelial growth of this plant pathogenic fungus after placing active mycelial plugs of each strain on Petri dishes using a disc diffusion method of EOs. The Minimum Inhibitory Concentration (MIC) of each EO was also determined. The growth rates observed under different temperatures and pH levels varied among isolates and did not show similar responses for the same levels of the two growth parameters. However, the maximum mycelial growth of the majority of isolates was reached between 20 and 30°C as well as at pH 7. Among the EOs tested, the results revealed that EO of *Thymus vulgaris* and *Origanum compactum* were very effective on controlling *R. solani* strain RS1 with growth inhibition rate of 75,9% and 60,6%, respectively. The MIC of these EOs was at 0.03%. According to the gas chromatography analysis, Carvacrol (61.8%) and thymol (47.8%) are the major constituents of *O. compactum* and *T. vulgaris* EOs, respectively, suggesting that these natural compounds have important potential to control *R. solani* and could be useful for developing effective organic fungicides.

Keywords: *Rhizoctonia solani*, Antifungal activity, Essential oil, Temperature, pH

INTRODUCTION

Rhizoctonia solani Kuhn (Teleomorph: *Thanatephorus cucumeris*) is one of the most serious soil-borne fungal pathogens, causing disease on a large number of host plant species (**Sneh *et al.*, 1991**). Critical disease symptoms caused by this pathogen, on host plants, include Root, stem and crown rot and damping-off (**Lahlali and Hijri, 2010; Naher *et al.*, 2014**). Many crops of economic importance worldwide are frequently affected by *R. solani* including potato, bean, tomato, soybean, strawberry and tulip, (**Kotba *et al.*, 2018; Lahlali and Hijri, 2010; Ismail & Ismail, 2011; Naher *et al.*, 2014**). Isolates of this pathogen has a complex biology and diversity in terms of myclial colour, size of aerial mycelium, growth rate, zonation, type and number of sclerotia, saprophytic behaviour, enzyme production and pathogenicity (**Hyakumachi *et al.*, 1988; Salunkhe *et al.* 2008**). In addition to the recognized methods to group isolates of *R. solani* taxonomically, diversity of growth character within different pH and temperatures levels has been noted by **Chang (1985)**. The most favorable temperature level for *R. solani* development is 23-28°C, while lower and higher optima have been noted for different isolates (**Abe, 1978**). Another report showed the optimal temperature between 25 to 35°C with the most convenient media were potato-dextrose-agar, malt-extract and Sabouraud-glucose (**Csöndes *et al.*, 2007**). In Morocco, no detailed information is available on the variability in *R. solani* species, in particular the diversity of isolates and their behavior under different pH and temperature regimes.

Chemical fungicides are often used to avoid the substantial yield losses caused by *R. solani*. However, the intensive use of chemical fungicides has not only created problems of fungicide resistance, but has also resulted in the increase of the soil contamination, high toxicity of microbial communities and a deterioration effect on the ozone layer (**Huang *et al.*, 2011**). In addition, chemical control is not completely effective, and *Rhizoctonia* disease still damages diverse crops (**Goudjal *et al.*, 2014**). To overcome this challenge, the use of plants extracts may be an alternative to conventional fungicides to control *R. solani*. The activity pathway of these biopesticides against fungi is unrevealed but may be interrelated to their general capability to soften or otherwise dislocate the reliability of cell membranes and cell walls (**Isman, and Machial, 2006**). The objectives of this study were to i) demonstrate the existing diversity among *R. solani* isolates based on growth at different pH and temperature regimes on culture media, ii) determine the efficacy of some plant-derived EOs against the growth of *R. solani* in laboratory assays, iii) identify the major constituents of these oils by gas chromatography method using appropriate detectors.

MATERIAL AND METHODS

Rhizoctonia solani isolates

Naturally diseased plants of different crops (melon, water melon, sugar beet, bean, olive tree, strawberries, potato and tomato) showing typical symptoms of root rot disease were collected from several locations in Morocco (Tab1). For fungal isolation, the collected plant roots were washed carefully under running tap water followed by sterile water, then dried between two filter papers. Roots were cut into small pieces, that were transferred into ethanol 70° during 3 min for surface sterilization. Surface sterilized pieces were then washed several times with sterilized water to wash out the remaining disinfectant solution. The pieces were then dried on sterilized filter papers. Using sterilized forceps, plot dried pieces were then transferred into Petri dishes containing potato dextrose agar medium (PDA) (**Rashad *et al.*, 2012**). Concerning potato tubers, sclerotia (black scurf) detached from tubers were disinfected in ethanol 70° for 1 min, then washed with sterile distilled water and dried on sterile filter paper. Disinfected sclerotia were divided in many pieces and left to culture on PDA medium (**Djébali *et al.*, 2014**). The dishes were then incubated at 25±1°C, and the fungal growth were checked two days after incubation. In order to obtain pure cultures, purification of the isolates was done using the hyphal tip technique (**Rashad *et al.*, 2012**). Identification of the pure isolates were done according to the cultural properties, in particular morphological and microscopical characteristics (**Sneh *et al.*, 1991**).

Table 1 Host origin and plant part studied of *R. solani* isolates

Strain code	Host plant	Host location (City)	Plant part studied
RS1	Tomato	Menasra (Kenitra)	Collar
RS3	Melon	Menasra (Kenitra)	Root system
RS4	Sugar beet	Berkane	Tuber
RS5	Bean	Agadir	Root system
RS6	Olive tree	Marrakech	Root system
RS7	Strawberries	Kenitra	Root system
RS8	Potato	Berkane	Tuber
RS9	Potato	Agadir	Tuber
RS10	Potato	Agadir	Tuber
RS11	Potato	Fes	Tuber
RS12	Potato	Agadir	Tuber
RS13	Potato	Agadir	Tuber
RS14	Potato	Agadir	Tuber
RS15	Potato	Rabat	Tuber
RS16	Potato	Casablanca	Tuber
RS17	Water melon	Laouamra (Larache)	Root system

Effect of temperature on mycelial growth of *R. solani* isolates

Different temperature levels 5; 10; 15; 20; 25; 30; 33 and 35°C were evaluated in this trial. Fifteen milliliters of PDA media were added into each of the Petri dish (9 cm diameter) then an agar plug of fungal inoculums (4 mm diameter) was cut aseptically with a sterile scalpel from the margin of 2 days old culture of the sixteen *R. solani* isolates. One disc was placed in the center of individual Petri dish and three dishes were used for each isolate. After inoculation, the Petri dishes were incubated maintaining every time one of the eight temperature levels. Radial mycelial growth was calculated by averaging the two diameters taken at right angle of each colony when mycelial growth of some isolates reached the edge of the Petri dish (**Goswami *et al.*, 2011**).

Effect of pH on mycelial growth of *R. solani* isolates

Five different levels of pH "5; 6; 7; 8 and 8.5" were maintained to study the isolates mycelial growth variation at different pH levels adjusted by adding HCl or NaOH before solidifying the PDA media (**Goswami *et al.*, 2011**). The method followed for inoculation of mycelial discs of *R. solani* isolates with five pH levels was the same as described before. After inoculation, the Petri plates were incubated at 25±1°C. Radial mycelial growth was calculated by averaging the two diameters taken at right angles for each colony when mycelial growth of some isolates reached the edge of the Petri dish.

In order to facilitate the conduct of the other tests, eight representatives isolates were selected from the sixteen isolates. This selection was based on the origin (plant) of isolates and the average growth rates (mm/d) of the sixteen isolates under different temperature and pH levels. The eight isolates selected were RS1, RS3, RS4, RS5, RS6, RS7, RS8 and RS17.

Extraction of essential oils from aromatic and medicinal plants

Aromatic and medicinal plants used in this research are listed in Tab 2. The plant material was collected from three different locations in Morocco then was dried under the shade condition. The EO was extracted by hydrodistillation of the dried plants, using a Clevenger-type apparatus. The EOs were then collected by decantation, dried using anhydrous sodium sulfate, weighted, and stored in a dark glass bottle at 4°C (**Negahban *et al.*, 2007**).

Table2 Medicinal and aromatic plants studied

Common name	Scientific name	Botanical family	Plant part used
Cistus	*Cistus ladanifer* L.	*Cistaceae*	Leaves
Lavander	*Lavandula stoechas* L.	*Lamiaceae*	Flowers
Oregano	*Origanum compactum* Benth.	*Lamiaceae*	Leaves
Rosemary	*Rosmarinus officinalis* L.	*Lamiaceae*	Leaves
Thyme	*Thymus vulgaris* L.	*Lamiaceae*	Leaves
Eucalyptus	*Eucalyptus sp.*	*Myrtaceae*	Leaves
Sage	*Salvia sp.*	*Lamiaceae*	Leaves

Antifungal activity evaluation

Petri dishes with 9 cm diameter and containing 20 ml of PDA media were used to evaluate the antifungal activity of EOs by the disc diffusion method (**Duru *et al.*, 2003**). Sterile Whatman paper discs of 6 mm diameter were placed on the PDA media, equidistant and near the border, where the EO (5 µL/disc) was added separately. An agar plug of fungal inoculums (6 mm diameter) was removed from a young culture of the eight fungal strains studied, and placed near the other border of the Petri dishes. The plates were incubated at 25±1ºC for 5 days, until the growth in the control plates reached the edge of the plates. Concerning the control, 5 µL of sterilized water was added to each disc. For each treatment, plates were prepared in triplicate and the percentage of growth inhibition was calculated using the following formula (**Topps and Wain 1957**):

Inhibition (%) = [A-B/A] ×100

A: Radial growth of control (mm)
B: Radial growth of treatment (mm)

Determination of Minimum Inhibitory Concentrations (MICs)

The MIC values of *R. solani* isolates were detremined following the agar dilution method as defined previously by **Gul *et al.* (2002)**. Appropriate volume of the EO was added aseptically to sterile molten PDA medium to produce the concentration range of 0,01% - 0,5%. After vortexing, the resulting PDA solutions were immediately poured into Petri plates. An agar plug of fungal inoculums (6 mm diameter) was removed from a young culture of all the fungal isolate tested, and placed in the center of the Petri dishes. The plates were prepared in triplicate for each treatment and were incubated at 25±1ºC for 5 days, until the growth in the control plates (PDA not mixed with EOs) reached the edge of the plates. The MIC values were determined as the lowest concentrations of the EO where the absence of growth was recorded.

Characterization of essential oils by Gas Chromatography analysis

Determination of the EOs chemical composition was done using a Hewlett-Packard model HP6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a DB-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm; Agilent Technologies, USA) and coupled to an HP model 5973 mass selective detector. The column temperature is programmed from 50 to 300 °C with an increase of 7 °C/min. The injector temperature was 290 °C. Purified helium was used as a carrier gas with a flow rate of 1 mL/min, and the split ratio was 60:1. Mass spectra (MS) was obtained, in EI mode, at 70-eV ionization energy and the mass range was from 35 to 400 m/z. 10 µL of each EO was diluted in 990 µL of pure hexane, and for the analysis 1 µL was injected. The apparatus was piloted by an HP electronic system equipped with a ChemStation Software "G1701BA, version B.01.00", and the data were analysed out with the same software. After each compound analysis, the Kovats retention index (RI) was calculated comparing to a standard mix of n-alkanes between C8 and C26 (Sigma-Aldrich Co.) analyzed under same conditions. The components were identified by comparing the IR and MS spectra with those reported in the literature (**Adams 2007, Habbadi *et al.*, 2018**) and by computer matching using standard reference databases (NIST98, Wiley275, and CNRS libraries).

Statistical analysis

Analysis of variance (ANOVA) was used to analyse results related to the effects of plant EOs on mycelial growth of *R. solani* strains and to check the existence of variability among *R. Solani* strains under different temperature and pH regimes on PDA media. Least Significant Difference (LSD) test was used at the 5% level of significance; Statistical analysis was performed using SAS statistic software.

RESULTS

Effect of temperature on mycelial growth of *R. solani* isolates

According to the ANOVA results, there is significant interaction between *R. solani* isolates and temperature (P<0.0001) and significant main effect of both temperature and isolates on growth rate (Tab 3). Comparison between the sixteen isolates showed that isolates don't develop under 5°C and 35°C except isolate RS7 under 35°C with the average growth rate (9.6 mm/d) (Table 4). Under temperatures 10°C, 15°C, 20°C, 25°C, 30°C and 33°C, there is a significant difference among isolates. Also, under 30°C, isolates RS1, RS3 and RS17 showed high average growth rate (13.2 mm/d for each isolate). Average growth rate revealed significant difference among different temperature levels for each isolate. For the majority of our isolates, there is no significant difference on the average growth rate between 20°C and 30°C. In addition, the growth rate started to decline and revealed very low values for temperatures above 30°C (Tab 4).

Table 3 Analysis of variance (ANOVA) on growth rate of *R. solani* isolates under temperature level effect

Source of variation	Degree of freedom	F-value	P-value
Isolate (I)	15	48.00	<.0001
Temperature (T)	7	758.14	<.0001
I × T	105	22.27	<.0001
Block	2	1.24	0.2913

Within column, means followed by same lower case letters are not significantly different based on the LSD.
Within row, means followed by same upper case letters are not significantly different on the LSD.

Table 4 Average growth rates (mm/d) of *R. solani* isolates under eight temperature levels

Isolate	5°C	10°C	15°C	20°C	25°C	30°C	33°C	35°C
RS1	0.0 Da	7.2 Cbc	2.0 Dh	9.3 BCa	12.7 Aba	13.2 Aa	10.1 ABCc	0.0 Db
RS3	0.0 Ea	7.2 CDbc	6.5 Ded	9.3 BCa	12.0 Aa	13.2 Aa	11.4 Aba	0.0 Eb
RS4	0.0 Da	7.2 Abc	0.0 Di	2.3 Cb	5.4 Bf	4.8 Bf	4.1 Bd	0.0 Db
RS5	0.0 Da	4.8 Ced	6.0 Cef	9.8 Ba	12.0 Aa	12.0 Aa	10.4 Bbc	0.0 Db
RS6	0.0 Da	7.2 Cbc	7.2 Cd	8.4 BCa	8.3 BCed	8.8 Bcd	10.1 Ac	0.0 Db
RS7	0.0 Ea	0.0 Ef	11.0 BCa	10.5 BCa	12.7 Aa	10.5 Cb	11.2 Bab	9.6 Da
RS8	0.0 Ea	6.0 Ccd	7.4 Bd	9.2 Aa	9.7 Ac	9.4 Abc	1.8 De	0.0 Eb
RS9	0.0 Ca	7.0 Bbc	8.9 Ac	8.2 ABa	9.0 Acd	8.0 ABd	0.0 Cf	0.0 Cb
RS10	0.0 Ea	4.0 Ce	5.0 Cf	7.6 Ba	12 Aa	8.0 Bd	0.0 Ef	0.0 Eb
RS11	0.0 Da	0.0 Df	6.7 Ced	10.3 Aa	7.8 Be	8.4 Bcd	0.0 Df	0.0 Db
RS12	0.0 Da	9.6 Aa	6.0 Bef	10.2 Aa	9.0 Acd	5.4 Bef	1.5 Ce	0.0 Db
RS13	0.0 Ea	7.2 Bbc	6.0 Cef	8.4 Aa	9.4 Ac	6.6 BCe	1.5 De	0.0 Eb
RS14	0.0 Ea	0.0 Ef	3.5 Dg	8.3 Ca	11.0 Ab	9.6 Bbc	0.0 Ef	0.0 Eb
RS15	0.0 Ea	7.3 Cbc	10.0 ABab	9.0 Ba	10.7 Ab	9.6 ABbc	1.5 De	0.0 Eb
RS16	0.0 Ea	8.3 Cb	9.0 BCbc	9.3 Ba	11.0 Ab	9.6 Bbc	4.8 Dd	0.0 Eb
RS17	0.0 Ba	0.0 Bf	2.0 Bh	10.7 Aa	12.0 Aa	13.2 Aa	11.0 Aab	0.0 Bb

Lowercase letter: Mean separation between different strains for each temperature.
Uppercase letter: Mean separation between different temperatures for each isolate.

Effect of pH on mycelial growth of *R. solani* isolates

Diagnostic of the ANOVA results (Tab 5) revealed significant interaction between *R. solani* isolates and pH level (P<0.0001) and significant main effect of both pH level and isolates on growth rate. Average growth rate showed significant difference among different pH levels for each isolate. All sixteen isolates of *R. solani* were able to develop on PDA medium at all levels of pH within the range of 5-8.5 with certain similarities between average growth rate under pH=7 and the control (Tab 6). The average growth rate also showed high value (20.0 mm/d) for the isolate RS1 under pH=7 and lower value (1.5mm/d) under pH=8.5 for the isolate RS10.

Table 5 Analysis of variance (ANOVA) on growth rate of *R. solani* isolates under pH level effect

Source of variation	Degree of freedom	F-value	P-value
Isolate	15	630.42	<.0001
pH	5	1699.98	<.0001
Isolate × pH	75	69.94	<.0001
Block	2	0.18	0.8395

Within column, means followed by same lower case letters are not significantly different based on the LSD.
Within row, means followed by same upper case letters are not significantly different based on the LSD.

Table 6 Average growth rates (mm/d) of *R. solani* isolates under five pH levels

Isolate	pH = 5	pH = 6	pH = 7	pH = 8	pH = 8.5
RS1	15.2 Da	16.2 CDb	20.0 Aa	18.6 Ba	11.3 Ea
RS3	14.2 Cab	19.3 Aa	19.0 Ab	17.8 Bb	9.7 Dd
RS4	6.4 Bh	7.3 ABj	8.3 Ak	6.3 Bk	4.0 Ce
RS5	14.8 Ba	13.5 Cd	19.8 Aab	14.2 BCc	10.6 Dbc
RS6	10.8 Aefg	10.4 Ag	10.5 Ai	10.9 Af	10.3 Ac
RS7	11.2 ABef	7.8 Cj	12.0 Ag	7.19 Cj	11.0 Bab
RS8	11.1 Bef	9.1 CDi	14.8 Acd	8.8 Di	3.2 Ef
RS9	10.2 Bfg	11.4 Aef	11.8 Agh	11.2 Af	2.2 Ch
RS10	10.3 Cfg	9.8 Ch	13.1 Af	11.2 Bf	1.5 Di
RS11	12.8 Bcd	11.7 Cef	9.6 Dj	12.8 Bd	4.4 Ee
RS12	9.7 Dg	14.4 Ac	11.1 Chi	11.9 Be	2.6 Egh
RS13	10.4 Defg	11.3 Cf	13.5 Aef	9.7 Eh	2.2 Fh
RS14	11.6 Cde	11.6 Cef	14.1 Bde	10.4 Eg	11.1 Dab
RS15	10.6 Cefg	11.9 Be	14.1 Ade	12.5 Bd	4.0 De
RS16	10.6 Eef	14.4 Bc	13.0 Cf	11.4 Def	3.1 Ffg
RS17	13.1 Cbc	11.6 Def	15.3 Ac	13.8 BCc	10.6 Dbc

Lowercase letter: Mean separation between different isolate for each pH.
Uppercase letter: Mean separation between different pH for each isolate.

Antifungal activity

The ANOVA results (Tab 7) revealed significant interaction between isolates and EO (P<0.0001) and significant main effect of both EO and isolates on growth inhibition rate. The growth inhibition rates of eight *R. solani* isolates using seven EOs of *Salvia sp*., *Cistus ladanifer* L., *Lavandula stoechas* L., *Thymus vulgaris* L., *Origanum compactum* Benth., *Rosmarinus officinalis* L. and *Eucalyptus sp.* are shown in Figure1. The growth inhibition rate showed high value for the isolate RS1 (75.9%) using the EO of *Thymus vulgaris* L. and the lower value was for the isolate RS4 (1.9%) using the EO of *Rosmarinus officinalis L.* EO of *Origanum compactum* showed the higher level of growth inhibition rates followed by *Thymus vulgaris* L. EO on all *R. solani* isolates except the RS1 isolate, suggesting the great potential of these two EOs in controlling the development of all *R. solani* isolates tested in this study. However, EO of *Cistus ladanifer* L. showed the lowest level of growth inhibition rates on most of *R. solani* isolates (Figure 2).

Table7 Analysis of variance (ANOVA) of growth rate of *R. solani* isolates under EOs effect

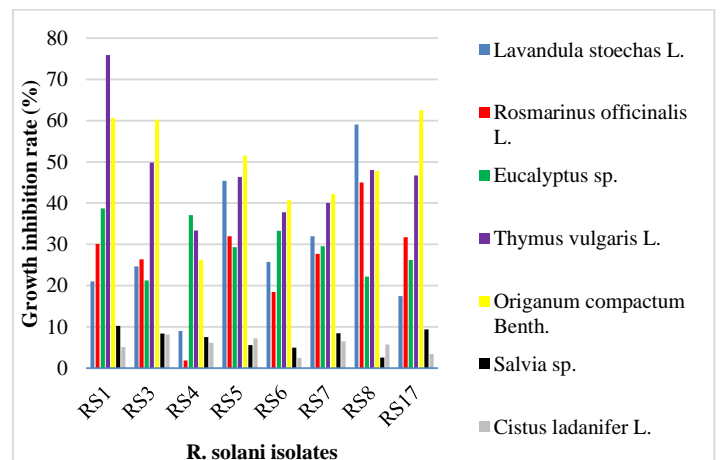| Source of variation	Degree of freedom	F-value	P-value
Isolate (I)	7	2319.51	<.0001
EO	6	25409.3	<.0001
I × EO	42	1035.34	<.0001
Block	2	2.34	0.1010

Figure1 Growth inhibition rates of *R. solani* isolates using plant Eos
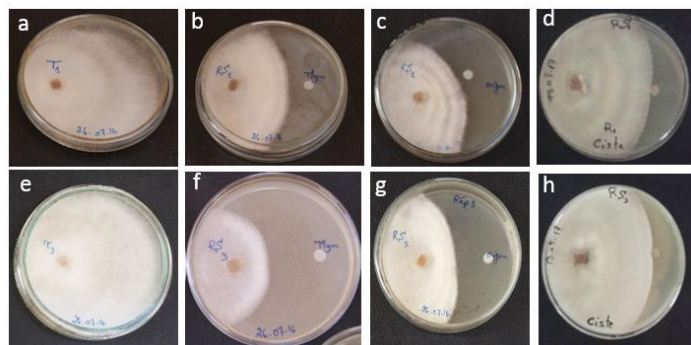
Figure 2 Mycelial growth inhibition of *R. solani* isolates RS1 (a,b,c,d) and RS3 (e,f,g,h) compared with control (a,e) using EOs of Thyme (b,f), Oregano (c,g) and Cistus (d,h)

Figure 3 Mycelial growth inhibition of *R. solani* RS5 and RS1 compared to the control (a, e) using EOs of *T. vulgaris* and *O. compactum* with different concentrations: 0.01% (b;f), 0.02% (c;g) and 0.03% (d;h)

Determination of Minimum Inhibitory Concentrations (MICs)

Results of this assay showed that the plant EOs have different MIC on *R. solani* isolates. However, the MIC of *T. vulgaris* and *O. compactum* EOs on *R. solani* isolates was similar with an average of 0.03% (Figure 3). Whereas, the others EOs showed a MIC higher than 0.15%. This difference could be due to the chemical compounds of each plant EO.
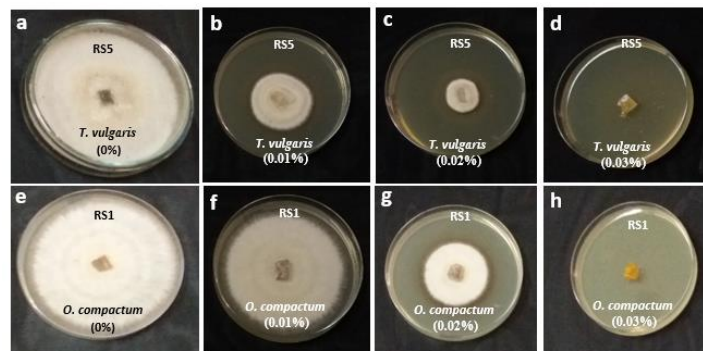
Essential oil composition

In this study, the GC-MS analysis revealed that the major compounds of the EOs examined were monoterpene hydro- carbons and phenolic monoterpenes. However, GC-MS analyses of *R. officinalis, O. compactum, T. vulgaris, R. officinalis* and *Eucalyptus* EOs conducted to the identification of different constituents (Tab 8). The major constituents of *O. compactum* EO were carvacrol (61.78%), γ-terpinene (12.46%), p-cymene (8.21%), and thymol (7.01%). While *T. vulgaris* EO was composed primarily of thymol (47.79%), p-cymene (24.13%), and γ-terpinene (8.19%). *R. officinalis* and *Eucalyptus* EOs were mainly composed of Cineole with concentrations of 46.28% and 67.08% respectively.

Table 8 GC-MS data for EO components identified

Compound	% of essential oils			
	Rosmarinus officinalis	*Thymus vulgaris*	*Origanum compactum*	*Eucalyptus sp.*
Thujene	-	0.57	0.92	-
α-Pinène	11.94	0.54	0.59	3
Camphene	4.76	0.16	0.08	-
Isoamylisovalerate	-	-	-	0.47
β-Pinène	3.05	0.11	0.12	0.24
Octen-3-ol	0.2	0.32	1.17	-
3-Octanone	-	0.06	0.10	-
Myrcène	1.35	1.34	1.43	-
3-Octanol	-	0.06	-	-
α-phellandrène	0.45	0.16	0.22	-
δ-3-Carene	-	0.07	0.08	-
Cinéole (Eucalyptol)	**46.28**	-	-	**67.08**
Ocimène-Cis-β	-	-	0.13	-
α-Terpinène	0.98	1.72	1.85	-
γ-Terpinène	1.32	8.19	12.46	0.94
Terpinolène	0.6	0.10	0.08	-
Pulégone	-	-	-	1.62
ρ-Cymene	1.58	24.13	8.21	11.51
Limonène	-	0.38	0.23	-
Cis-Linalool Oxide	-	0.09	-	-
Fenchyl-alcool	-	-	-	0.25
Linalool	1.81	3.06	0.86	-
β-fenchyl alcohol	-	-	-	3.12
p-Cymenène	-	0.07	-	-
Bicyclo 3,1,1-hept-3-en-2-ol	-	-	-	0.86
1,8-Cineole	-	0.12	-	-
Bicyclo 2,2,1-hept-3-one	-	-	-	0.24
Camphor	12.32	0.05	-	0.34
Pinocamphone	0.2	-	-	-
Pinocarvone	0.23	-	-	-
Bornéol	4.49	0.45	0.14	0.51
Iso-pinocamphone	0.28	-	-	-
Thujol	-	-	-	0.55
Terpinen-4-ol	1.14	0.59	0.42	1.01
Cis-Dishydro Carvone	-	0.06	0.06	-
α-Terpinéol	4.1	-	-	-
Verbenone	0.6	-	-	-
Bornyl acétate	1.38	-	-	-
Thymol Méthyl Ether	-	0.04	-	-
Carvacrol Méthyl Ether	-	0.05	-	-

Carvacrol	-	5.03	**61.78**	0.75
Thymol	-	**47.79**	7.01	0.53
(E)Caryophyllène	-	1.76	1.47	-
Trans-Caryophyllène	0.75	-	-	0.26
α-Humulène	-	0.06	0.06	-
α-Selinène	-	0.04	-	-
α-Murolène	-	0.01	-	-
β-Bisabolène	-	0.05	0.13	-
γ-Cadinène	-	0.07	0.03	-
δ-Cadinène	-	0.16	0.06	-
Caryophyllène Oxide	-	0.80	-	-
Isoaromadendrène	-	-	-	0.27
Aromadendrène	-	-	-	0.95
Viridiflorol	-	-	-	0.43
γ-gurjunène	-	-	-	2.28
δ- gurjunène	-	-	-	0.35
Total	99.81	98.35	98.89	97.56

DISCUSSION AND CONCLUSION

Diseases induced by *R. solani* are challenging because this fungi survives for many years as mycelium or as sclerotia in organic matter or soil under different conditions and has an important host range (**Ismail & Ismail, 2011**). Similar results to our current study have also been reported by many other investigators (**Chang, 1985; Dubey *et al*., 2012; Jaaffar *et al.*, 2016**). For *R. solani*, the most favorable temperature regime was from 25 to 35°C (**Csöndes *et al*., 2007**), also **Chang (1985)** found that both mycelial growth and sclerotial developpement of *R. solani* were maximal at pH 7 which confirm the results of our study. **Sharma and Chowdhury (1984)** observed that *R. solani* has a low incidence in cauliflower at neutral pH comparing to pH 7.4 - 8.5. Also, **Marcelo and Vega (1988)** found that the most favorable pH for *R. solani* development was in pH 6-6.5. Moreover, **Kobayashi (1985)** found that soil pH has a direct influence on the hyphal growth of *R. solani*.

In the last several years, many researches have been conducted for the development of safer antifungal agents such as EOs and plants extracts to control plant pathogens in agriculture (**Bajpai and Kang, 2010**). The EOs derived from medicinal and aromatic plants have been reported to show interesting antimicrobial effects against fungi, bacteria and viruses (**Reichling *et al.*, 2009**). **Katooli *et al.* (2011)** evaluated the effect of EOs for suppressing the mycelial growth of *R. solani*. The antifungal activity of thyme EO has well proved against fungi such as *R. solani* (**Zambonelli *et al.*, 1996**) and in our current study, both EOs of *T. vulgaris* and *O. compactum* showed the highest level of growth inhibition rates on *R. solani* isolates.

In the present study, among the EOs that were selected for GC analysis, the carvacrol and thymol were identified as main constituents for *O. compactum* and *T. vulgaris*, respectively. It is well known that some compounds that are able to inhibit microbial growth (**Naovi *et al*., 1991**). Additionally, antagonistic or synergistic effects between some EOs constituents may also affect the observed antimicrobial activity of these EOs. **Didry *et al*. (1993)** reported a synergistic activity of thymol and carvacrol against some bacteria.

Origanum compactum and *T. vulgaris* are two aromatic and medicinal plants belonging to the Laminacea family, which is a source for many plants with therapeutic benefits. These plants are largely distributed in north and east of Morocco and commonly used as spices and condiments (**Zantar *et al.*, 2014**). The chemical composition of *O. compactum* and *T. vulgaris* EOs has been described by different authors (**Imelouane *et al.*, 2009; Govaris *et al*., 2011**). An important diversity has been demontrated; however, carvacrol and thymol remain the major constituents. According to **Di Pasqua *et al.* (2007)** thymol can damage the citrate metabolic pathway and influence the enzymes involved in ATP synthesis. Also, carvacrol showed impact on cell membrane structure by increasing the fluidity and permeability of the membrane.

The use of these EOs tested on *R. solani* fungus may be a good source of new alternative active ingredient for an effective and sustainable management of this disease. Also, EOs have two importants advantages; low toxicity for people and environment due to their natural characteristics and low risk for resistance development by pathogenic microorganisms. However, further formulation and field experiments are necessary to achieve this goal.

REFERENCES

Abe, H. (1978). Anastomosis groups of isolates of *Rhizoctonia solani* Kuhn from potatoes. *Bull Hokkaido Pref Agric Exp Stn*, *40*, 61-70.

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry* (Vol. 456). Carol Stream, IL: Allured publishing corporation.

Bajpai, V. K., & Kang, S. C. (2010). Antifungal activity of leaf essential oil and extracts of Metasequoia glyptostroboides Miki ex Hu. *Journal of the American Oil Chemists' Society*, *87*(3), 327-336. https://doi.org/10.1007/s11746-009-1500-6

Chang, Y. C. (1985). Effect of temperature, pH and water potential on mycelial growth and sclerotial formation of *Rhizoctonia solani* AG 1. *Chung-hua nung yeh yen chiu= Journal of agricultural research of China*. 34 (4): 454- 463.

Csöndes, I., Kadlicskó, S., & Gáborjányi, R. (2007). Effect of different temperature and culture media on the growth of Macrophomina phaseolina. *Communications in agricultural and applied biological sciences*, *72*(4), 839-848.

Di Pasqua, R., Betts, G., Hoskins, N., Edwards, M., Ercolini, D., & Mauriello, G. (2007). Membrane toxicity of antimicrobial compounds from essential oils. *Journal of agricultural and food chemistry*, *55*(12), 4863-4870. https://doi.org/10.1021/jf0636465

Didry, N. P., Dubreuil, L., & Pinkas, M. (1993). Antibacterial activity of thymol, carvacrol and cinnamaldehyde alone or in combination. *Die Pharmazie*, *48*(4), 301-304.

Djébali, N., Elkahoui, S., Taamalli, W., Hessini, K., Tarhouni, B., & Mrabet, M. (2014). Tunisian *Rhizoctonia solani* AG3 strains affect potato shoot macronutrients content, infect faba bean plants and show *in vitro* resistance to azoxystrobin. *Australasian Plant Pathology*, *43*(3), 347-358. https://doi.org/10.1007/s13313-014-0277-8

Dubey, S. C., Tripathi, A., & Upadhyay, B. K. (2012). Molecular diversity analysis of *Rhizoctonia solani* isolates infecting various pulse crops in different agro-ecological regions of India. *Folia microbiologica*, *57*(6), 513-524.https://doi.org/10.1007/s12223-012-0165-y

Duru, M. E., Cakir, A., Kordali, S., Zengin, H., Harmandar, M., Izumi, S., & Hirata, T. (2003). Chemical composition and antifungal properties of essential oils of three Pistacia species. *Fitoterapia*, *74*(1), 170-176.

Goswami, B. K., Rahaman, M. M., Hoque, A. K. M. A., Bhuiyan, K., & Mian, I. H. (2011). Variations in different isolates of Rhizoctonia solani based on temperature and pH. *Bangladesh Journal of Agricultural Research*, *36*(3), 389-396. https://doi.org/10.3329/bjar.v36i3.9267

Goudjal, Y., Toumatia, O., Yekkour, A., Sabaou, N., Mathieu, F., & Zitouni, A. (2014). Biocontrol of *Rhizoctonia solani* damping-off and promotion of tomato plant growth by endophytic actinomycetes isolated from native plants of Algerian Sahara. *Microbiological Research*, *169*(1), 59-65. https://doi.org/10.1016/j.micres.2013.06.014

Govaris, A., Botsoglou, E., Sergelidis, D., & Chatzopoulou, P. S. (2011). Antibacterial activity of oregano and thyme essential oils against Listeria monocytogenes and Escherichia coli O157: H7 in feta cheese packaged under modified atmosphere. *LWT-Food Science and Technology*, *44*(4), 1240-1244. https://doi.org/10.1016/j.lwt.2010.09.022

Gul, H. I., Ojanen, T., & Hänninen, O. (2002). Antifungal evaluation of bis Mannich bases derived from acetophenones and their corresponding piperidinols and stability studies. *Biological and Pharmaceutical Bulletin*, *25*(10), 1307-1310.https://doi.org/10.1248/bpb.25.1307

Güllüce, M., Sökmen, M., Şahin, F., Sökmen, A., Adigüzel, A., & Özer, H. (2004). Biological activities of the essential oil and methanolic extract of Micromeria fruticosa (L) Druce ssp serpyllifolia (Bieb) PH Davis plants from the eastern Anatolia region of Turkey. *Journal of the Science of Food and Agriculture*, *84*(7), 735-741. https://doi.org/10.1002/jsfa.1728

Habbadi, K., Meyer, T., Vial, L., Gaillard, V., Benkirane, R., Benbouazza, A., ... & Lavire, C. (2018). Essential oils of *Origanum compactum* and *Thymus vulgaris* exert a protective effect against the phytopathogen Allorhizobium vitis. *Environmental Science and Pollution Research*, *25*(30), 29943-29952. https://doi.org/10.1007/s11356-017-1008-9

Huang, X., Zhang, N., Yong, X., Yang, X., & Shen, Q. (2012). Biocontrol of *Rhizoctonia solani* damping-off disease in cucumber with Bacillus pumilus SQR-N43. *Microbiological Research*, *167*(3), 135-143. https://doi.org/10.1016/j.micres.2011.06.002

Hyakumachi, M., Mushika, T., Ogiso, Y., Toda, T., Kageyama, K., & Tsuge, T. (1998). Characterization of a new cultural type (LP) of *Rhizoctonia solani* AG2-2 isolated from warm-season turfgrasses, and its genetic differentiation from other

cultural types. *Plant Pathology*, *47*(1), 1-9.https://doi.org/10.1046/j.1365-3059.1998.00212.x

Imelouane, B., Amhamdi, H., Wathelet, J. P., Ankit, M., Khedid, K., & El Bachiri, A. (2009). Chemical composition and antimicrobial activity of essential oil of thyme (Thymus vulgaris) from Eastern Morocco. *Int. J. Agric. Biol*, *11*(2), 205-208.

Ismail, A. E. W. A., & Ismail, M. M. (2011). Antagonistic activity of some fungi and cyanobacteria species against *Rhizoctonia solani*. *International Journal of Plant Pathology*, *2*(3), 101-114. DOI: 10.3923/ijpp.2011.101.114

Isman, M. B., & Machial, C. M. (2006). Pesticides based on plant essential oils: from traditional practice to commercialization. *Advances in phytomedicine*, *3*, 29-44. https://doi.org/10.1016/S1572-557X(06)03002-9

Jaaffar, A. K. M., Paulitz, T. C., Schroeder, K. L., Thomashow, L. S., & Weller, D. M. (2016). Molecular characterization, morphological characteristics, virulence, and geographic distribution of Rhizoctonia spp. in Washington State. *Phytopathology*, *106*(5), 459-473.https://doi.org/10.1094/PHYTO-09-15-0208-R

Katooli, N., Maghsodlo, R., & Razavi, S. E. (2011). Evaluation of eucalyptus essential oil against some plant pathogenic fungi. *Journal of Plant Breeding and Crop Science*, *3*(2), 41-43.

Kobayashi, W.H. (1985). Natural suppression of soil borne plant diseases. Plant protection Bulletion, *Taiwan* 27(3). 17 1-178.

Kotba, I., Achouri, M., Benbouazza, A., Touhami, A. O., & Douira, A. (2018). Morphological, Pathogenic and Molecular Characterisation of *Rhizoctonia solani* strains isolated from Potato. *Annual Research & Review in Biology*, 1-16.. https://doi.org/10.9734/ARRB/2018/44926

Lahlali, R., & Hijri, M. (2010). Screening, identification and evaluation of potential biocontrol fungal endophytes against *Rhizoctonia solani* AG3 on potato plants. *FEMS microbiology letters*, *311*(2), 152-159.

Naher, L., Ali, M. A., Dey, T. K., Islam, M. M., & Ismail, A. (2014). Evolution of disease and potential biocontrol activity of Trichoderma SP. against *Rhizoctonia solani* on potato. *Bioscience Journal*, *30*(4).

Naovi, S. A., Khan, M. S., Vohora, S. B., & Naqvi, S. (1991). Antibacterial, anti-fungal and anthelmintic investigations on Indian medicinal plants.

Negahban, M., Moharramipour, S., & Sefidkon, F. (2007). Fumigant toxicity of essential oil from Artemisia sieberi Besser against three stored-product insects. *Journal of stored products research*, *43*(2), 123-128.

Rashad, Y. M., Abdel-Fattah, G. M., Hafez, E. E., & El-Haddad, S. A. (2012). Diversity among some Egyptian isolates of *Rhizoctonia solani* based on anastomosis grouping, molecular identification and virulence on common bean. *African Journal of Microbiology Research*, *6*(37), 6661-6667. DOI: 10.5897/AJMR12.109

Reichling, J., Schnitzler, P., Suschke, U., & Saller, R. (2009). Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties–an overview. *Complementary Medicine Research*, *16*(2), 79-90..DOI:10.1159/000207196.

Salunke, V. N., Armarkar, S., & Ingle, R. W. (2008). Efficacy of fungicides and antagonistic effect of bio-agent *Rhizoctonia bataticola* isolates. *Annals of Plant Physiology*, *220*(1), 134-137.

Sneh B, Burpee L, Ogoshi A. (1991). Identification of *Rhizoctonia* species, pp. 133. American Phytopathological Society Press, Saint Paul, USA. https://www.cabdirect.org/cabdirect/search/?q=bn%3a%22089054123X%22

Topps, J. H., & Wain, R. L. (1957). Investigations on fungicides. III. The fungitoxicity of 3-and 5-alkyl-salicylanilides and para-chloroanilides. *Annals of Applied Biology*, *45*(3), 506-511. https://doi.org/10.1111/j.1744-7348.1957.tb05888.x

Zambonelli, A., d'Aulerio, A. Z., Bianchi, A., & Albasini, A. (1996). Effects of essential oils on phytopathogenic fungi *in vitro*. *Journal of Phytopathology*, *144*(9-10), 491-494. https://doi.org/10.1111/j.1439-0434.1996.tb00330.x

Zantar, S., Yedri, F., Mrabet, R., Laglaoui, A., Bakkali, M., & Zerrouk, M. H. (2014). Effect of *Thymus vulgaris* and *Origanum compactum* essential oils on the shelf life of fresh goat cheese. *Journal of Essential Oil Research*, *26*(2), 76-84. https://doi.org/10.1080/10412905.2013.871673