Moroccan Journal of Chemistry
ISSN: 2351-812X
http://revues.imist.ma/?journal=morjchem&page=login
Chibane & al. / Mor. J. Chem. 8 N°2 (2020) 456-465

Antifungal activity of *Asteriscus graveolens* (Forssk.) Less essential oil against *Fusarium oxysporum* f. sp. *albedinis*, the causal agent of "Bayoud" disease on date palm

E. Chibane[(a)], A. Essarioui[(b)], M. Ouknin[(a)], A. Boumezzourh[(a)], A. Bouyanzer[(c)], L. Majidi[(a)]*

[(a)]*Laboratory of Natural Substances, Molecular Synthesis and Dynamics, Faculty of Science and Technics, Errachidia, Morocco*
[(b)]*National Institute for Agricultural Research, Regional Center, Errachidia, Morocco*
[(c)]*Laboratoire de Chimie Analytique Appliquée Matériaux et Environnement (LCA2ME URAC18), Faculté des Sciences, B.P. 717, 60000 Oujda, Morocco*

* *Corresponding author:* lmajidi@yahoo.fr
Received 22 Jan 2020, Revised 20 April 2020, Accepted 27 April 2020.

Abstract

Date palm (*Phoenix dactylifera* L.) is an important food source and commercial perennial crop in the Sahara and North Africa. "Bayoud", a vascular wilt disease, incited by the soil borne fungal pathogen *Fusarium oxysporum* f. sp. *albedinis* (FOA), has become a serious threat to date production in date palm-growing regions in Morocco. Substantial body of research reported antifungal properties of a wide range of plant extracts. The present work aims to determine chemical composition and anti-FOA activity of *Asteriscus graveolens* (Forssk.) Less (AG) essential oil. GC/GC-MS analysis revealed the presence of forty-two distinct chemical components in the essential oil samples. The main compounds of the studied essential oils were 6-Oxocyclonerolidol (7.7–73.8%), cis-8-acetoxychrysanthenylacetate (0.6–50.1%) cis-chrysantenyl acetate (0.3–15.4%), 6-hydroxycyclonerolidol (2–13.3%), τ-cadinol (0.4–12.2%) and α-oxobisabolene (0.2–5.5%). Mycelial growth and spore germination of FOA were found to be strongly inhibited by the oil when tested using agar dilution assay. The lowest essential oil (EO) concentration that inhibits 100% of mycelial growth was 3500 ppm for leaves and 4000 ppm for aerial parts EO. These findings contribute significant insight into the potential of using plant extracts for controlling "Bayoud" disease.

Keywords: *Asteriscus graveolens (Forssk.) Less; Antifungal activity; "Bayoud" disease; Fusarium oxysporum f. sp. Albedinis; Phoenix dactylifera L*

1. Introduction

Date palm, *Phoenix dactylifera* L., is a plant that plays an essential role in the oasis regions. In Morocco, until a few years ago, date palm had been cultivated in traditional groves located primarily along two major wadies in the southern part of the country. Date palm traditional plantations in Morocco are estimated to include more than five million trees planted over 48000 ha. In recent years, significant efforts have been made by the government of Morocco, as part of the Green Moroccan Plan, to encourage through financial supports to investors the establishment of new and modern date palm plantations outside the traditional orchards. As a result, thousands of hectares of date palm plantations with advanced management technologies have been established in expansion areas to date. Other similar investments are in progress for an expected total acreage of more than 20 000 ha of modern date palm plantations by the year 2020. "Bayoud" disease is the principal enemy of palm trees that puts at stake the future of these investments and threatens date industry in Morocco [1-2]. "Bayoud" is a vascular wilt disease incited by the soil borne fungal pathogen FOA. The pathogen penetrates the tree through the roots and progresses in the vascular system and ultimately kills palms. The disease first appeared in the Darâa valley-Morocco in 1890. However, the causal agent had not been identified until 1930. In the meantime, the disease had swept across date-producing areas in Morocco and destroyed more than 10 million palm trees [3]. Qualitatively speaking, the disease has wiped out many good cultivars and is still threatening others. The disease was spread mainly by flood irrigation water along the valleys, and the transplantation of symptomless diseased plant materials (offshoots) over long distances. The disease can also be transmitted to a neighboring tree by root contact. Unfortunately, the best Moroccan commercial varieties such as "Mejhool" and "Boufeggous" are highly susceptible and require intensive management to survive the attack of the pathogen [1-2]. Attempts to control the disease using systemic fungicides have been unsuccessful because of technical, economic and environmental reasons. Instead, the tremendous genetic diversity that characterizes palm trees in the Moroccan traditional groves has offered great opportunities for mass selection for genetic resistance against the pathogen. Multiple oases were deeply explored and individual trees with good fruit quality and thriving in hot spots of the disease were identified as candidate resistant genotypes. Offshoots were taken from these trees and tested for resistance using artificial inoculation techniques in experimental stations. Genotypes with confirmed resistance and good fruit quality have been multipli4ed, along with susceptible but highly valued varieties, using tissue culture techniques. This consists of culturing meristematic cells for large scale production of pathogen-free palm "vitroplants" even if the plant material of origin is diseased. Mass selection and the development of date palm tissue culture techniques have enabled the selection and multiplication of new date palm varieties that are resistant to Bayoud disease. These varieties have been used to restore areas that have been devastated by the disease in traditional groves and to support management strategy in the new plantations outside the valleys. However, most modern plantations grow "Mejhool" variety that is highly prized in local and international markets for the large size, the sweet taste and the juicy flesh of its dates. A negative consequence of this intensification of date production coupled with genetic uniformity in cultivars is an anticipated increase in vulnerability to diseases caused by microbial pathogens. One possible for sustainable management of the disease is the use of plant derivative antimicrobial proprieties. For example, it has been reported that extracts of aromatic and medicinal plants have antifungal effects [4-6] and can be used as an alternative to synthetic fungicides to control plant fungal diseases because of their environmental safety and less negative effects on human health [7]. The *Asteraceae* is a large family of plants, which include more than 23.000 species [8]. The genus *Asteriscu*s species are important for their uses in traditional medicine. Effectively, we found in the literature that species of that genus possess antibacterial, diuretic, antifungal, antispasmodic, anti-inflammatory activities. They are also known for treating, gastrointestinal tract complaints, bronchitis, cephalic pains and fever [9-

10]. Recently our laboratory has already highlighted the antifungal effect of the EO from AG on postharvest phytopathogenic fungi of apples [11]. The purpose of our study was to evaluate the antifungal activity of EO extracted from AG against FOA by evaluating its mycelial radial growth and spore germination inhibition.

2. Material and Methods

2.1. Plant material

The aerial parts of the plant were harvested in April 2017 during the flowering season in Merroutcha (N 31.5540716°; W 4.8858036°, southeast Morocco) using a pruning shear to allow its regeneration. They were then dried in the shade and powdered using a hammer mill.

2.2. Fungi material

FOA strain used was isolated from the spine of a date palm tree, affected by Bayoud, originating from the province of Errachidia and identified by Dr. Adil ESSARIOUI at the Regional Centre of the Institute of Agronomic Research in Errachidia. A stock of FOA has been prepared for use in the various tests envisaged.

2.3. EO extraction and analysis

EO was extracted using a Clevenger apparatus, dried under anhydrous sodium sulfate and stored in an amber bottle at 4 °C. The EOs extracted from leaves, stems, flowers and whole plant were prepared and analyzed by GC/GC-MS while keeping a part for the tests carried out. This analysis was carried out using a Perkin Elmer Autosystem (Walhton, MA, USA) XL, equipped with a single injector and two flame ionization detectors (FID) for compound detection. The apparatus was used for simultaneous sampling with two fused silica capillaries (60 m x 0.22 mm, film thickness 0.25 µm) with different stationary phases, respectively polar (Rtx-Wax, polyethylene glycol) and non-polar (Rtx-1, polydimethyl siloxane). The carrier gas is helium with a column head pressure of 25 psi (in the published article it is the flow rate that is mentioned 1ml/min). The temperature of the injector and detector is 280 °C. The temperature programming consists of an increase from 60 to 230 °C, at 2 °C/mn, then in a 30-minute step at 230 °C. Injection was done in split mode with a division ratio of 1/50. The volume of EO injected is 0.1 µl. For each compound, the retention indices (Ir) are calculated from the retention times of a standard range of n-alkanes (C5-C30) with linear interpolation, using the equation of Van Den Dool and Kratz [12] and the Perkin Elmer software.

2.4. Antifungal activity

2.4.1. Mycelial radial growth inhibition

The evaluation of radial mycelial growth inhibition was performed according to the method described by El Ouadi et al. [13]. Different EO concentrations were prepared using an aqueous suspension of 0.2% agar and the Czapek culture medium (2000 ppm, 2500 ppm, 3000 ppm, 3500 ppm and 4000 ppm). The control was done with a Petri dish containing the sterilized Czapek culture medium and 1ml of the 0.2% agar suspension. These culture media were left in the laminar hood for 24 hours to ensure that they were not contaminated. A 7 mm diameter FOA mycelial disc was removed using a sterilized cork borer from the peripheral part of a 7-day FOA colony and placed in the center of each Petri dish. The preparations were then incubated under 28 °C. The assessment of mycelial growth was done by measuring the largest and smallest diameter of the colony using an electronic caliper. The diameter of the colony being the average of two diameters. Measurements were taken when a colony reached the edge of the Petri dish. The inhibition percentage was calculated using the formula (1) [14]:

$$I(\%) = \left(\frac{D_c - D_t}{D_c}\right) \times 100 \quad (1)$$

Where D_C represents the diameter of the colony in the control test and D_t represents the diameter of the colony corresponding to the different EO concentrations tested.

2.4.2. Spore germination inhibition

Spores were collected from the peripheral area of a 7-day-old colony of FOA and used to prepare a suspension of 20 spores/ml. 1ml of the latter was spread in Petri dishes containing the Czapek medium and different concentrations of the prepared EOs. The preparations were then incubated under 28 °C. The control was done using a Petri dish containing the Czapek medium and 1ml of the 0.2% agar suspension. The counting of the colonies was done after 4 days. The various tests were carried out in triplicate. The germination inhibition percentage was calculated according to the formula (2) [15].

$$I\ (\%) = \left(\frac{S_C - S_t}{S_C}\right) \times 100 \quad (2)$$

Where S_C is the number of spores that germinated in the control box and S_t the number of spores that germinated in different tested mediums with different EO concentrations.

2.5. Statistical analysis

The descriptive statistical parameters were calculated with the software package SPSS version 22.0 for Windows. Pearson's correlation coefficient analysis, Principal Component Analysis (PCA), and Cluster Analysis (CA) are the most common multivariate statistical methods used to identify the relationship among the studied EOs [16-17]. In the study, PCA was performed with Varimax rotation with Kaiser Meyer-Olkin normalization (KMO = 0.46). Clustering and calculations were performed using the Ward's method and by the Euclidean distances [16].

3. Results and discussions

3.1. EO extraction kinetic and yield

Figure 1 represents AG different parts EO extraction kinetic. The yield of EO from leaves was 0.66%, followed by aerial parts 0.62%, flowers 0.46% and stems 0.37%. These values are higher than those obtained by Cristofari et al., which were 0.65% for the leaves, 0.31% for the flowers and 0.11% for the stems [9]. As shown in Figure 1, the optimal extraction time is 4 hours.

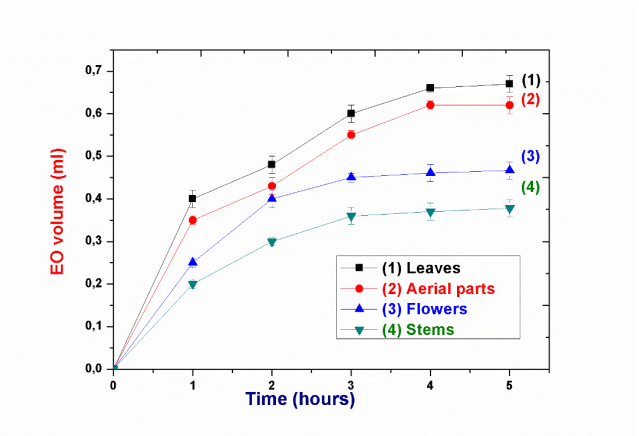

Figure 1. *Asteriscus graveolens* essential oil extraction kinetic.

3.2. EO analysis

The forty-two compounds detected (Table 1) belong to oxygenated sesquiterpenes (78.9–93.8%) and oxygenated monoterpenes (3.2–17.6%) which are the main groups of constituents followed by sesquiterpene hydrocarbons (0.6–1.8%) and monoterpene hydrocarbons (0.1–1.5%) in EOs from distinct parts of AG. 6-Oxocyclonerolidol is the major component in EO from leaves (73.8%), aerial part (72.5%) and stems (52.2%). However, cis-8-acetoxychrysanthenylacetate (50.1%) is the main compound of flowers EO and less represented in the other parts. Other compounds were detected with low concentrations such as cis-Chrysantenyl acetate (0.3–15.4%), 6-Hydroxycyclonerolidol (2–13.3%), τ-Cadinol (0.4–12.2%) and α-Oxobisabolene (0.2–5.5%). The detected compounds constitute 96.7% to 98.4% of the studied EOs. Cristofari et al. reported that the main compounds of EO from the same species were 6-Oxocyclonerolidol (74.9%), cis-8-Acetoxychrysanthenyl acetate (48.5%), cis-Chrysanthenyl acetate (13.4%) and 6-Hydroxycyclonerolidol (12.2%) [9]. Another study conducted by Chaib et al., showed that the major component of EO from AG were cis-chrysanthenyl acetate (31.1%), myrtenyl acetate (15.1%), and kessane (11.5%) [7]. Differences observed in chemical composition of EO can be attributed to ecological factors [9].

3.3. Descriptive statistics

3.3.1. Pearson's correlation analysis

Obtained correlation coefficients are presented in Table 2 as a linear correlation matrix. It shows intensively significant correlations which were observed between (cis-Chrysantenyl acetate, γ-Cadinene, and cis-Chrysantenol, $r < 0.9$), (γ-Cadinene, cis-Chrysantenol, and cis-Chrysantenyl acetate, $r < 0.9$), (τ-Cadinol, γ-Cadinene, and cis-8-Acetoxychrysanthenylacetate, $r < 0.9$), (6-Hydroxycyclonerolidol, and Humulene epoxide II, $r = 0.999$) and a significantly positive correlation was also detected between α-Oxobisabolene, cis-8-Acetoxychrysanthenylacetate and 6-Hydroxycyclonerolidol, $r = 0.998$. A significantly positive correlation at $p < 0.05$ was found between the carvacrol and 6-methylhept-5-en-2-one ($r = 0.957$). On the other hand, a highly positive correlation was found between τ-Cadinol, cis-Chrysantenol and cis-Chrysantenyl acetate ($r < 0.9$), α-Oxobisabolene, cis-Chrysantenol, cis-Chrysantenyl acetate, and γ-Cadinene ($r < 0.95$, $p < 0.05$).

3.3.2. Cluster analysis

The results of hierarchical clustering for the studied EOs are presented in Figure 2. Two distinct clusters with different associations were distinguished. The first cluster formed by parts L (Leaves), AP (Aerial parts) and S (Stems) is characterized by 6-Oxocyclonerolidol (50.4-74.9%), 6-Hydroxycyclonerolidol (8.8-12.2%), Intermodeol (3.1-4.3%) and Humulene epoxide II (1.4-1.8%). The second cluster formed by part F (Flowers) is characterized by cis-8-Acetoxychrysanthenyl acetate (48.5%), cis-Chrysantenyl acetate (13.4%), τ-Cadinol (11.4%), 6-Oxocyclonerolidol (7.8%) and α-Oxobisabolene (5.7%), that allows separating the flowers from the other groups.

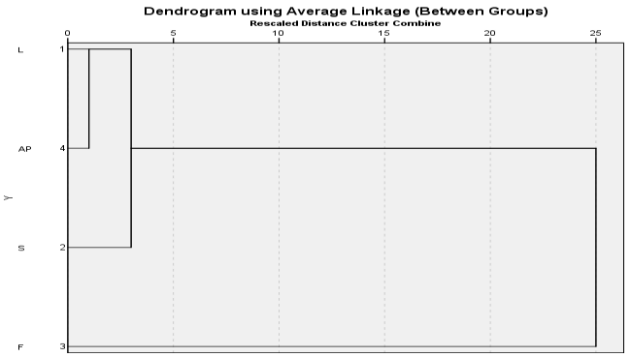

Figure 2. Dendrogram results from Ward method of hierarchical cluster analysis for the four parts of *Asteriscus graveolens* on the basis of similarity.

Table 1. Chemical profile of *Asteriscus graveolens* (Forssk.) Less EO.

N[a]	Components	[b]Ir apl	[c]Ir pl	[d]Leaves (L)	[d]Stems (S)	[d]Flowers (F)	[d]Aerial Parts (AP)
1	α-pinene	933	1004	0.1	0.1	0.1	1.2
2	6-methylhept-5-en-2-one	980	1302	0.1	0.5	-	0.3
3	Myrcene	982	1145	tr	-	-	tr
4	1,8-Cineole	1023	1183	0.1	0.1	-	0.1
5	Linalool	1081	1544	0.6	0.6	0.4	0.2
6	α-Thujone	1090	1384	0.1	-	-	-
7	α-Campholenal	1108	1446	0.1	-	-	-
8	Camphor	1124	1470	0.4	-	-	0.6
9	cis-Chrysantenol	1146	1445	-	0.2	0.5	-
10	Albene	1152	1270	-	1	0.1	-
11	Terpinen-4-ol	1162	1580	0.2	0.2	0.2	0.1
12	α-Terpineol	1170	1700	0.2	0.1	0.2	0.2
13	Estragole	1177	1650	-	-	-	0.2
14	Carvotanacetone	1218	1664	0.6	0.8	0.3	0.2
15	cis-Chrysantenyl acetate	1248	1526	**0.3**	**6.4**	**15.4**	**2.1**
16	Carvacrol	1279	2135	0.6	1.5	0.6	0.9
17	α-Copaene	1384	1476	-	-	0.2	-
18	Longifolene	1414	1562	tr	tr	-	tr
19	trans-Caryophylene	1418	1579	-	-	0.1	-
20	trans-α-Bergamotene	1431	1572	-	-	0.2	-
21	Sesquisabinene	1435	1642	-	-	0.2	-
22	epi-β-Santalene	1442	1629	-	-	tr	-
23	α-Humulene	1452	1628	0.1	0.3	0.1	0.3
24	Alloaromadendrene	1458	1620	-	-	0.1	-
25	γ-Muurolene	1470	1680	-	tr	-	tr
26	Germacrene D	1474	1700	-	-	0.1	-
27	β-Bisabolene	1502	1754	-	-	0.1	-
28	γ-Cadinene	1504	1737	0.2	0.3	0.5	0.2
29	δ-Cadinene	1518	1705	0.1	0.3	0.3	0.1
30	(Z)-Nerolidol	1522	1518	0.2	0.2	-	0.4
31	Kessane	1528	1715	0.4	0.5	-	0.5
32	α-Cadinene	1530	1731	-	-	tr	-
33	6-Oxocyclonerolidol	1555	1969	**73.8**	**52.2**	**7.7**	**72.5**
34	cis-8-Acetoxychrysanthenylacetate	1564	2122	**0.6**	**8.5**	**50.1**	**2.1**
35	Caryophyllene oxide	1576	1921	0.2	0.8	0.2	0.1
36	Humulene epoxide II	1598	1975	1.5	1.7	0.2	1.3
37	6-Hydroxycyclonerolidol	1622	2246	**12**	**13.3**	**2**	**9.9**
38	τ-Cadinol	1631	2102	**0.4**	**2.9**	**12.2**	**0.3**
39	β-Eudesmol	1636	2160	1.2	1.4	0.4	0.2
40	α-Cadinol	1639	2221	-	-	0.2	-
41	Intermodeol	1643	2160	3.3	3.4	0.4	2.5
42	α-Oxobisabolene	1707	2266	0.2	1	**5.5**	0.2
	Monoterpene hydrocarbons			**0.2**	**0.6**	**0.1**	**1.5**
	Oxygenated monoterpenes			**3.2**	**9.9**	**17.6**	**4.6**
	Sesquiterpene hydrocarbons			**0.4**	**0.9**	**1.8**	**0.6**
	Oxygenated sesquiterpenes			**93.8**	**85.9**	**78.9**	**90**
	Total identified			**97.6**	**97.3**	**98.4**	**96.7**

[a]Order of elution are given on apolar column (Rtx-1); [b]Ira = retention indices on the apolar column (Rtx-1); [c]Irp = retention indices on the polar column (Rtx-Wax); [d]Relative percentages of components (%) are calculated on GC peak areas on the apolar column (Rtx-1) except for components with identical RIa (concentration are given on the polar column); - : not detected; tr: Traces (<0.05%).

Table 2. Pearson's correlation matrix among the variable.

Compounds	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
α -pinene (1)	1																			
6-methylhept-5-en-2-one (2)	.225	1																		
Linalool (3)	-.870	.118	1																	
Camphor (4)	.778	-.025	-.522	1																
cis-Chrysantenol (5)	-.494	-.366	.037	-.823	1															
Carvotanacetone (6)	-.666	.505	.917	-.464	-.115	1														
cis-Chrysantenyl acetate (7)	-.391	-.354	-.075	-.770	.993**	-.207	1													
Carvacrol (8)	.000	.957*	.246	-.314	-.100	.599	-.101	1												
γ-Cadinene (9)	-.471	-.425	.000	-.786	.998**	-.171	.993**	-.167	1											
δ-Cadinene (10)	-.577	.130	.302	-.962*	.855	.314	.831	.408	.816	1										
Kessane (11)	.420	.789	.073	.513	-.859	.381	-.852	.594	-.891	-.485	1									
6-Oxocyclonerolidol (12)	.453	.430	.022	.773	-.996**	.192	-.995**	.175	-1.000**	-.808	.894	1								
cis-8-Acetoxychrysanthenylacetate (13)	-.376	-.568	-.124	-.655	.964*	-.336	.970*	-.339	.981*	.689	-.951*	-.984*	1							
aryophyllene oxide (14)	-.469	.740	.625	-.642	.143	.841	.093	.883	.074	.631	.328	-.058	-.123	1						
Humulene epoxide II (15)	.124	.746	.377	.340	-.805	.628	-.834	.598	-.844	-.388	.951*	.853	-.932	.470	1					
6-Hydroxycyclonerolidol (16)	.079	.727	.419	.316	-.794	.657	-.828	.586	-.833	-.376	.935	.843	-.924	.481	.999**	1				
τ-Cadinol (17)	-.432	-.527	-.059	-.712	.981*	-.263	.981*	-.285	.992**	.738	-.938	-.994**	.997**	-.049	-.902	-.891	1			
β-Eudesmol (18)	-.679	.409	.946	-.377	-.192	.987*	-.291	.480	-.240	.196	.381	.262	-.386	.743	.642	.675	-.318	1		
Intermodeol (19)	.048	.669	.450	.335	-.810	.660	-.850	.525	-.846	-.415	.915	.857	-.929	.441	.993**	.997**	-.897	.691	1	
α-Oxobisabolene (20)	-.400	-.574	-.099	-.666	.966*	-.318	.969*	-.343	.982*	.692	-.955*	-.984*	0.998**	-.116	-.928	-.918	.998**	-.365	-.922	1

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

3.3.3. Principal Component Analysis (PCA)

PCA is a method that allows the simplification of data by studying relationships among all variables to determine similarities and dissimilarities among individuals. Analysis of the links between chemical composition of the EOs from AG (whole plant, leaves, stems and flowers) was performed using this method and only the discriminant variables were taken into account.According to the results obtained (Figure 3), we notice that the elements forming the EOs of different parts represent a variability of 99.99% on the three axes, of which the first represents 57.09%, 22.85% for the second axis and 20.50% for the third axis.

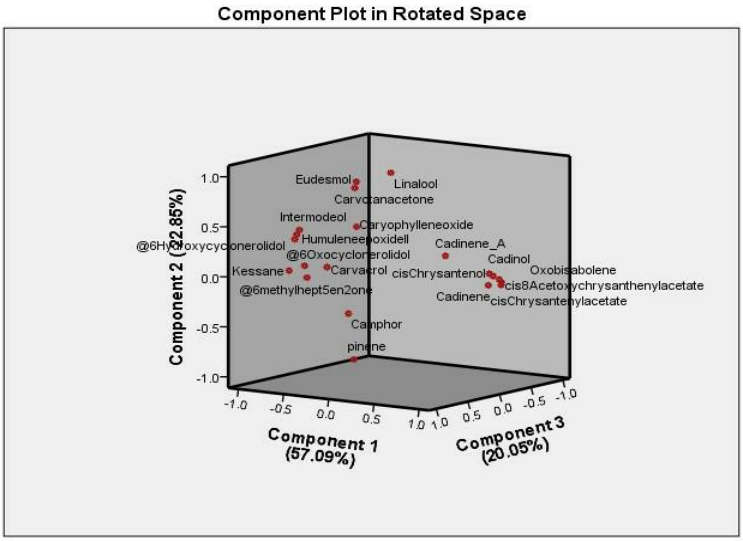

Figure 3. Plot of loading of three principal components in PCA results.

3.4. Antifungal activity

3.4.1. Radial growth inhibition

Table 3 represents radial growth inhibition percentage of different concentrations of EOs from AG. The obtained results show that the inhibition percentage increases with increasing of EO concentration. It goes from 22.50% to 100% and depends on the AG used part. The lowest concentrations of EOs from leaves and aerial parts that suppressed completely (I = 100%) the growth of the fungi are 3500 ppm and 4000 ppm respectively. The found antifungal activity can be attributed to the AG chemical composition especially the main compounds (cis-8-acetoxychrysanthenyl acetate, cis-chrysantenyl acetate, 6-hydroxycyclonerolidol, τ-cadinol and α-oxobisabolene) or the latter in synergy with other components [18]. Indeed, previous studies have shown that compounds contained in AG have an antifungal effect such as intermedeol, β-eudesmol, τ-cadinol and nerolidol [9, 19].

Table 3. Inhibition percentage of different essential oils concentrations against *Fusarium oxysporum* f.sp. *albedinis.*

	2000 ppm	2500ppm	3000ppm	3500ppm	4000ppm
Leaves	53.31%	68.10%	86.60%	100.00%	100.00%
Aerial parts	43.13%	63.53%	75.33%	86.66%	100.00%
Stems	29.94%	41.81%	53.97%	62.35%	84.66%
Flowers	22.50%	28.55%	32.06%	35.41%	42.51%

3.4.2. Spore germination inhibition

To our knowledge, no study has been published concerning the inhibition of FOA spore germination using AG essential oils. Table 4 shows the results of spore germination inhibition test. It shows that the inhibition is dose-dependent. Indeed, it increases with increasing of EO concentration. It ranges from 6.67% to 71.67% and depends on AG used part. The results of the named test can be assigned to the major components of the studied EOs or to a synergism between them and other compounds [18]. Effectively, the EO from leaves, which has a high content of these compounds, exhibits a high effect than EOs from other parts.

Table 4. Inhibition percentage of spore germination of different essential oils concentrations against *Fusarium oxysporum* f.sp. *albedinis.*

	2000ppm	2500ppm	3000ppm	3500ppm	4000ppm
Leaves	38.33%	48.33%	63.33%	68.33%	71.67%
Aerial parts	26.67%	41.67%	53.33%	61.67%	63.33%
Stems	18.33%	26.67%	28.33%	41.67%	51.67%
Flowers	6.67%	8.33%	18.33%	18.33%	25.00%

The spore germination inhibition which has been revealed can be attributed to the major compounds of the EO, namely cis-8-acetoxychrysanthenyl acetate, cis-chrysantenyl acetate, 6-hydroxycyclonerolidol, τ-cadinol and α-oxobisabolene and/or other compounds detected in the studied EO like α-cadinol, β-eudesmol, τ-cadinol, α-humulene, and caryophyllene oxide. Effectively, Su and Ho reported that these latter showed an antifungal activity [20]. In addition, Zouari et al., found that cis-chrysantenyl acetate and α-thujone exhibit a strong antifungal activity [21].

4. Conclusion

GC/GC-MS analysis of AG essential oil revealed the presence of four chemical groups namely oxygenated sesquiterpenes, oxygenated monoterpenes, sesquiterpene hydrocarbons and monoterpene hydrocarbons. These groups include the detected forty-two compounds. The statistical analysis of compounds contained in EOs from different parts of AG showed that chemical profile depends on the part of the plant used. Using the methods of radial growth and spore germination inhibition, the studied EOs showed a strong antifungal activity against FOA, this activity can be assigned to the major compounds (6-Oxocyclonerolidol, cis-8-acetoxychrysanthenyl acetate, cis-Chrysantenyl acetate, 6-Hydroxycyclonerolidol, τ-Cadinol, and α-Oxobisabolene) acting alone or in synergy with other less represented compounds. Finally, the antifungal activity found contributes significant insight into the potential of using AG extracts for controlling "Bayoud" disease.

References

[1] A. Essarioui, M.H. Sedra, Lutte contre la maladie du bayoud par solarisation et fumigation du sol. Une expérimentation dans les palmeraies du Maroc, *Cah. Agric.*, 26 (2017) 45010.

[2] F. Jaiti, A. Meddich, I. El Hadrami, Effectiveness of arbuscular mycorrhizal fungi in the protection of date palm (Phoenix dactylifera L.) against bayoud disease, *Physiol. Mol. Plant Pathol.*, 71 (2007) 166–173.

[3] M.A. Mazri, R. Meziani, Micropropagation of Date Palm: A Review, *Cell. Dev. Biol.* 4(3) (2015) 160.

[4] N. Hasheminejad, F. Khodaiyan, M. Safari, Improving the antifungal activity of clove essential oil encapsulated by chitosan nanoparticles, *Food Chem.*, 275 (2019) 113–122.

[5] C. M. Bedoya-Serna, G.C. Dacanal , A.M. Fernandes, S.C. Pinho, Antifungal activity of nanoemulsions encapsulating oregano (*Origanum vulgare*) essential oil: in vitro study and application in Minas Padrão cheese, *Braz. J. Microbiol.*, 49 (2018) 929–935.

[6] M.F. Begum, M.F. Mahal, M.S. Alam, Inhibition of spore germination and mycelial growth of three fruit rot pathogens using some chemical fungicides and botanical extracts, *J. Life Earth Sci.*, 5 (2010) 23-27.

[7] D. Jasso de Rodríguez, F.A. Trejo-González, R. Rodríguez-García , M.L.V. Díaz-Jimenez, A. Sáenz-Galindo, F.D. Hernández-Castillo, J.A. Villarreal-Quintanilla, F.M. Pena-Ramos, Antifungal activity in vitro of Rhus muelleri against Fusarium oxysporum f. sp. Lycopersici, *Ind. Crops Prod.*, 75 (2015) 150–158.

[8] M. Mohammad hosseini, A. Venditti, Satyajit D. Sarker, Lutfun N.A. Akbarzadeh, Chemical composition of the essential oils and extracts of Achillea species and their biological activities: A review, *J. Ethnopharmacol.*, 199 (2017) 257–315.

[9] G. Cristofari, M. Znini, L. Majidi, H. Mazouz, P. Tomi, J. Costa, J. Paolini, Chemical Diversity of Essential Oils from Asteriscus graveolens (Forssk.) Less.: Identification of cis-8-Acetoxychrysanthenyl Acetate as a New Natural Component, *Chem. Biodivers.*, 9 (2012) 727-738.

[10] F. Chaib, H. Allali, M. Bennaceur, G. Flamini, Chemical Composition and Antimicrobial Activity of Essential Oils from the Aerial Parts of Asteriscus graveolens (FORSSK.) LESS. And Pulicaria incisa (LAM.) DC.: Two Asteraceae Herbs Growing Wild in the Hoggar, *Chem. Biodivers.*, 14 (2017) e1700092.

[11] M. Znini, G. Cristofari, L. Majidi, H. Mazouz, P. Tomi, J. Paolini, J. Costa, Antifungal Activity of Essential Oil from *Asteriscus graveolens* against Postharvest Phytopathogenic Fungi in Apples, *Nat. Prod. Commun.*, Vol. 6 (11) 2011.

[12] H.Van Den Dool, P. Dec Kratz, A Generalization of the Retention Index System Including Linear Temperature Programming Gas-Liquid Partition Chromatography, *J. Chromatogr.*, 11 (1963) 463–471.

[13] Y.E. Ouadi, M. Manssouri, A. Bouyanzer, L. Majidi, H. Bendaif, H. Elmsellem, M.A. Shariati, A. Melhaoui, B. Hammouti, Essential oil composition and antifungal activity of Melissa officinalis originating from north-Est Morocco, against postharvest phytopathogenic fungi in apples, Microb. Pathog., 107 (2017) 321-326.
[14] D.K. Pandey, N.N. Tripathi, R.D. Tripathi, S.N. Dixit, Fungitoxic and phytotoxic properties of the essential oil of Hyptis suaveolens Fungitoxische und phytotoxische Eigenschaften des ätherischen Öis von *Hyptis suaveolens*, *J. Plant. Dis. Protect.*, 89 (1982) 344-349.
[15] N. Gemeda, Y. Woldeamanuel, D. Asrat, A. Debella, Effect of essential oils on Aspergillus spore germination, growth and mycotoxin production: a potential source of botanical food preservative, *Asian Pac. J. Trop. Biomed.*, 4 (2014) S373-S381.
[16] M. Ouknin, A. Romane, M.Y. Arjouni, L. Majidi, Mineral Composition, multivariate analysis of some oligo-elements and heavy metals in some species of genus *Thymus*, *J. Mater. Environ., Sci.* 9 (2018) 980-985.
[17] X.W. Lu, L.J. Wang, L.Y. Li, K. Lei, L. Huang, D. Kang, Multivariate statistical analysis of heavy metals in street dust of Baoji, NW China. *J. Hazard Mater.*, 173 (2010) 744–749.
[18] C. Cabral, C. Cavaleiro, M.J. Gonçalves, M.T. Cruz, M.C. Lopes, L. Salgueiro, *Otanthus maritimus* (L.) Hoffmanns. & Link as a source of a bioactive and fragrant oil, *Ind. Crops Prod.*, 43 (2013) 484–489.
[19] M. Marino, C. Bersani, G. Comi. Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. *Int. J. Food Microbio.*, 67 (2001) 187–195.
[20] Y.C. Su and C.L. Ho, Composition of the Leaf Essential Oil of Phoebe formosana from Taiwan and its in vitro Cytotoxic, Antibacterial and Antifungal Activities. *Nat. Prod. Commun.*, 11 (2016) 845-888.
[21] S. Zouari, N. Zouari, N. Fakhfakh, A. Bougatef, M.A. Ayadi and M. Neffati, Chemical composition and biological activities of a new essential oil chemotype of Tunisian *Artemisia herba alba* Asso, *J. Med. Plant. Res.*, 4 (2010) 871-880.