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Evaluation of antifungal potential of *Ruta chalepensis* L. essential oil against *Mauginiella scaettae*, fungus responsible for the inflorescence rot of date palm (*Phoenix dactylifera* L.)

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Abstract

The objective of our work is to research for new natural bioactive products as an alternative means of control against the fungus responsible for the inflorescence rot of date palm *Mauginiella scaettae*. *In vitro* tests of the antifungal activity of essential oil of *Ruta schalepensis* were performed. The results showed that the yield of essential oil obtained by hydrodistillation of the aerial part of *R. chalepensis* was 0.41 ± 0.02%. Their chemical composition was analyzed by GC and GC/MS technique. 09 chemical compounds were identified, representing 99.99% of the total composition of the essential oil. The main constituents were camphor (76.57%), followed by borneol (8.75%), eucalyptol (3.12%), trans-4-Isopropyl-1-methyl-2-cyclohexen-1-ol (3.55%) and 1-Terpineol (2.34%). Three concentrations (0.25, 0.5 and 1) µl/ml PDA (potato dextrose agar) of the essential oil were used to study *in vitro* antifungal activity against *M. scaettae*. The results showed a significant effect on mycelial growth. The values of the inhibition rate vary between 47.88 ± 1.78% and 83.49 ±1.78%. In comparison between these three concentrations of essential oil, the two concentrations 0.5 and 1 µl/ml PDA have important antifungal capacity than the third concentration 0.25 µl /ml PDA. Our results indicated that the essential oil of *R. chalepensis* could be used as a biofungicide for fungal diseases of date palm.

1. Introduction

The date palm (*Phoenix dactylifera* L.) commonly called as date palm is a plant of great socio-economic interest. It is a foremost cultivated crop in the Middle East, Arabian and North African countries (Imad Uddin *et al*., 2020). In Algeria**,** the phoeniculture occupies a place of first rank in the Saharan agriculture (Benziouche and Cheriet, 2012). However, this culture meets immense difficulties in particular the pests, diseases and weeds. These pests and diseases cause considerable losses to production and can lead to genetic erosion (Dakhia *et al*., 2013). Among the diseases that infect date palms is inflorescence rot. This is a serious disease that can be epidemic in the wettest growing regions or in very wet years (Carpenter and Elmer, 1978). In North Africa, this disease (called Khamedj by the natives) has been reported in almost all date palm growing areas (Abdullah *et al*., 2005). The economic importance of this disease is attributed to considerable yield losses (Bounaga and Djerbi, 1990). The fungus, *M. scaettae* is considered the main cause of inflorescence rot. The causal fungus of this disease was reported for the first time by Cavara in Libya in 1925 (Abdullah *et al*., 2005). It is an imperfect fungus of the order Hyphales, with chains of hyaline conidia, fragmented into single or two-celled articles (Abdullah *et al*., 2005; Bounaga and Djerbi, 1990). In order to control this disease, various means of control have been adopted, including the removal of contaminated parts of the inflorescence and burning them immediately after harvest and the treatment of palms with various fungicides (Carpenter and Elmer, 1978; Chabrolin, 1930). The use of these fungicides can have harmful consequences on humans and their environment, due to toxicological and eco-toxicological risks. In this context, there is a major interest in natural extracts of medicinal plants such as essential oils as a means of biological control, given their antimicrobial activity which is linked to their chemical composition and in particular to the nature of their major volatile components (Bouatrous, 2019; Bouhlali *et al*., 2021; Caillet and Lacroix, 2007). Recently, many studies have focused on the biological and antifungal properties of herbal medicines (Parveen *et al*., 2020). Among the biological and antifungal properties studied cited the oils of essential oils and extracts of Rutaceae which are sources of various natural products with biological activities, including antifungal, antioxidant, depressant and anti-inflammatory (Merghache *et al*., 2009; Mejri *et al*., 2013; Aouadhi *et al*., 2013). Among the species belonging to the Rutaceae family, *Ruta chalepensis* L. commonly called Fidjel in Algeria. It is a spontaneous species, very abundant, it grows spontaneously in rock gardens, lawns, rocks and dry hills (Beniston, 1984); characterized by oval, large, pinnate and blue-green leaves which have numerous lanceolate oblong lobes. The inflorescence of this species is in the form of a cyme (Daoudi *et al*., 2016).

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The main objectives of this study were: (i) to determine the chemical composition of the essential oil of *R. chalepensis* and (ii) to investigate *in vitro* the antifungal activity of this oil against the mycelial growth of *M. scaettae*.

2. Materials and Methods

2.1 Plant material

The plant of *R. chalepensis* was collected in March 2019, in the commune of M'chounech (Wilaya of Biskra). It was identified in the Centre of Scientific and Technical Research on Arid Regions (CRSTRA) at the University of Biskra. The aerial part (stems and leaves) of the specie *R. chalepensis* was air-dried, away from light and then stored in clean paper bags until the time of extraction.

2.2 Essential oil extraction

The essential oil of *R. chalepensis* was extracted by hydrodistillation in a Clevenger-type. 100 g of dry plant material placed in a Pyrex glass flask, and extracted with 1000 ml of distilled water for 3 hours. The essential oil was collected and stored at 4°C away from light (Ismaily Alaoui *et al*., 2014).

2.2.1 Calculation of essential oil yield

The yield of essential oil was defined according to the Afnor standard (1986). It is expressed as a percentage and calculated by the following formula:

Rd = M'/M.100

Rd: The yield of essential oil (%)

M': the quantity of oil obtained (g)

M: the mass of dry plant material (g) (Afnor, 1986).

2.3 Gas chromatography-mass spectrometry (GC-MS) analysis

The analysis was carried out in a Trace 1310 gas chromatograph equipped with an ISQ single quadrupole mass spectrometer (Thermo Fisher Scientific, Austin, TX). The procedure was set to an initial temperament 60°C for 6/min, then ramp at 2°C/min to 230°C, and finally 30/min in 230°C. The ion source and detector temperature was 250°C and 250°C, respectively. Sample filtered with a 0.22/ µm disposable syringe filter. A volume of 1/µl was injected in split less model. Separation of sample was performed on a Thermo TG-WAXMS GC column (60/ m × 0.25/ mm ID × 0.25/ µm) using helium as carrier gas at 1.2/ ml/min. Mass spectral scan range was set at the rate of 55-550 (amu). Peak identification was conducted by comparison of the known components stored in the NIST Demo, Wiley7, Wiley9, redlip, mainlip, WinRI (Adams, 2001).

2.4 Fungal material

2.4.1 Isolation and purification of the pathogen

Small pieces (2 cm long) from infected inflorescence examined were surface-sterilized with 2% sodium hypochlorite for 3 min, rinsed three times for 3 min in sterile distilled water and blotted dry on sterilized filter paper. After these, fragments were dried on sterile pads and then placed on top of moist sterilized filter paper in Petri dishes, with 3 fragments per dish. Incubation took place at 25 ± 2°C for 7 days in the dark. Once well differentiated colonies, they will be then re-inoculated several times in new Petri dishes containing PDA medium for obtaining purified cultures. After purification, macro and microscopic observations were performed for identification of the fungi based on the morphological characteristics of the mycelium, and conidia (Rattan and Al-Dboon, 1980; Abdullah *et al*., 2005).

2.5 Antifungal activity of *R. chalepensis* essential oil

The evaluation of the antifungal activity of essential oil is performed by the direct contact method. Essential oil was diluted with Tween 20 (0.1% v/v) and then added into 20 ml PDA to obtain different final concentrations. Concentrations of 0.25, 0.5 and 1 µl /ml PDA were prepared. 1 ml of each concentration is added to each Petri dish containing 20 ml of PDA medium, and then stirred for 5 min in order to homogenize the medium PDA with essential oil. After solidification of the medium, A mycelial disk of 0.5 cm in diameter, cut from the periphery of a 7-day old culture, was inoculated in the centre of each PDA plate (9 cm diameter), and then incubated in the dark at 25 ± 2°C for 7 days. 20 ml PDA containing Tween 20 (0.1%) without essential oil was used as negative control. Three replicates were used for tested oil and control (Remmal *et al.*, 1993). A daily measurement of the radial growth diameter of each cultured explants was taken, by measuring the average of two perpendicular diameters passing through the centre of each dish. The inhibition rate and mycelial growth rate of each concentration is determined by the following formulas:

- **Inhibition rate (IR)**

  IR = [(L – 1) / L] x 100

  IR: Inhibition rate of mycelial growth (%);

  L: Diameter of the control colony (cm);

  1: Diameter of the colony in the experiment (cm); (Leroux and Credet, 1978).

- **Mycelial growth rate (MGR)**

  $$MGR = [D_1/T_1] + [(D_2 - D_1)/T_2] + [(D_3 - D_2)/T_3] + ... + [(D_n - D_{n-1})/ T_n]$$

  MGR = Speed of mycelial growth (cm/day)

  D = Diameter of the growth zone on each day (cm).

  T = Incubation time (Day); (Cahagnier and Richard-Molard, 1998).

2.6 Minimum inhibitory concentrations (MIC)

Minimum inhibitory concentration (MIC) of essential oil was determined according to the method reported by (Remmal *et al.*, 1993). It corresponds to the minimum concentration of essential oil for which no growth observed by naked eyes. Its determination was made by observing the total absence of the growth of the strains in the different concentrations of essential oil tested.

2.7 Statistical analysis

The results obtained were subjected to one-way analysis of variance (ANOVA), followed by Newman-Keuls test, and the means are represented as (mean ± SD). The results are significant when $p<0.05$, using the XLSTAT 2014.5.03 analysis software.

3. Results

3.1 Essential oil yield and chemical composition of *R. chalepensis*

The yield of essential oil obtained by hydrodistillation of *R. chalepensis* was 0.41 ± 0.02%. This oil had a dark yellow color, and a strong fetid odor.

The gas chromatography-mass spectrometry (GC-MS) analysis of extracted essential oil of *R. chalepensis* revealed 09 compounds representing 99.99% of the total chemical composition. The essential oil was characterized by high content of oxygenated monoterpens (97.38%) among which, camphor (76.57%), borneol (8.75%), eucalyptol (3.12%), trans-4-Isopropyl-1-methyl-2-cyclohexen-1-ol (3.55%) and 1-Terpineol (2.34%) were the most abundant. Else, the essential oil exhibited low amounts of monoterpens, dl-Limonene (1.56%), and p-Cymene (1.05%). The results of the GC-MS analysis are summarized in Table 1 and Figure 1.

Table 1: Chemical composition of the essential oil of *R. chalepensis*

No	Compound Name	RT	Area %
1	dl-Limonene	15.28	1.56
2	Eucalyptol	15.72	3.12
3	p-Cymene	19.37	1.05
4	Acetic acid, 2-ethylhexyl ester	31.95	1.35
5	Camphor	34.23	76.57
6	trans- 4-Isopropyl-1-methyl-2-cyclohexen-1-ol	37.13	3.55
7	3-Cyclohexen-1-ol,4-methyl-1-(1-methylethyl)	39.49	1.70
8	1-terpineol (Terpenene-1-ol)	40.99	2.34
9	Borneol	45.17	8.75
	Total		99.99

RT: retention time.

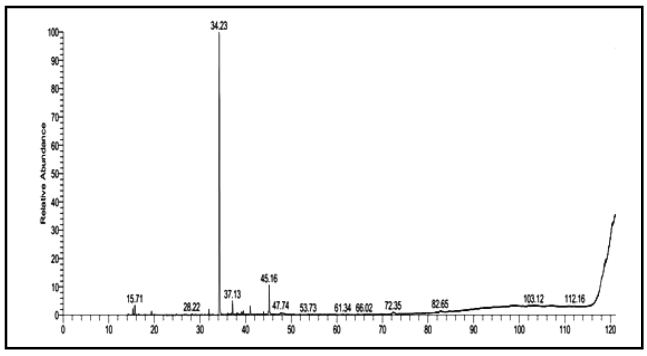


Figure 1: Chromatographic profile for GC-MS analysis of *R. chalepensis* essential oil.

3.2 Antifungal activity of essential oil

3.2.1 Inhibition rate

The essential oil of *R. chalepensis* was tested for antifungal activity against plant pathogenic fungi, *M. scaettae*. Our results showed that the inhibition rate is highly significant ($p \leq 0.001$) for different concentrations. The concentrations of essential oil (0.5 and 1) µl/ml PDA showed significant inhibition rate values respectively 78.12 ± 11.08% and 83.48 ± 1.78%, compared to the third concentration 0.25 µl/ml PDA 47.88 ± 1.78% (Figure 2).

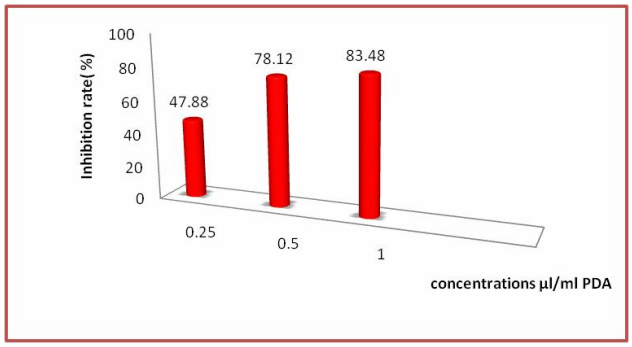


Figure 2: Inhibition rate of mycelial growth of the fungal strain tested by essential oil of *R. chalepensis*.

3.2.2 Mycelial growth rate (MGR)

The results of the antifungal assay of the essential oil extract of *R. chalepensis* showed that there is a very highly significant difference ($p<0.0001$). The concentrations of essential oil (0.25, 0.5 and 1 µl/ml PDA) lowered the fungal growth of *M. scaettae*. The lowest growth rate was observed at 0.5 and 1 µl/ml PDA concentrations where 0.54 ± 0.08 and 0.51 ± 0.01cm/day are recorded, respectively. Results of the separation of the growth rate mean show that no difference was observed between the two concentrations (0.5 and 1 µl/ml PDA). On the other hand, the highest growth rate was observed in the control (1.14 ± 0.15 cm/day) compared to the tested doses (Figure 3).

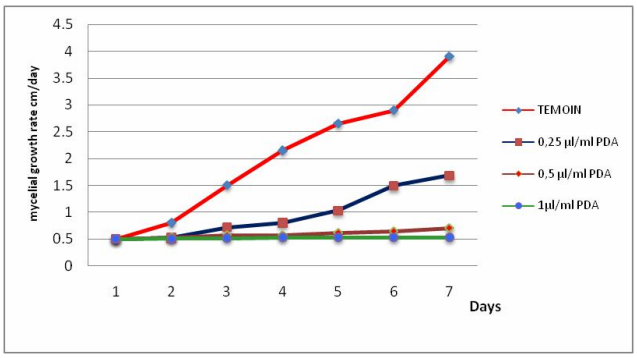
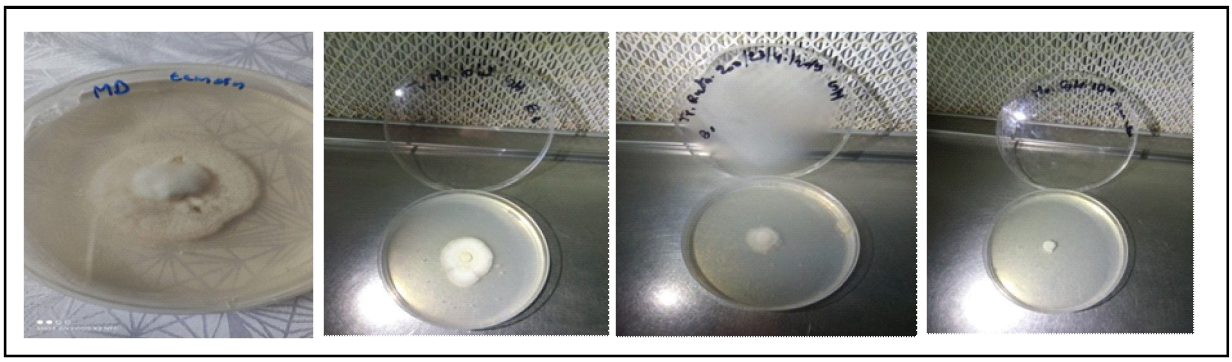


Figure 3: Evolution of the mycelial growth of *M. scaettae* according to the different concentrations of the essential oil of *R. chalepensis* during seven days of incubation.

3.3 Minimum inhibitory concentrations (MIC)

According to the results recorded in Figure 2 which presents the inhibition rate of mycelial growth of the fungal strain tested by essential oil of *R. chalepensis*. It has been shown that all the concentrations of essential oil applied (0.25, 0.5 and 1) µl/ml PDA partially inhibited the growth of the fungal strains tested where we recorded 47.88 ± 1.78%, 78.12 ±11.08% and 83.48 ±1.78%, respectively. The results obtained showed that *M. scaettae* exhibited some resistance where 100% inhibition was not achieved but considering the doses administered to the culture medium, this fungus remains susceptible to the action of the oil (Photo 1).



Control **0.25 µl/ml PDA** **0.5 µl/ml PDA** **1 µl/ml PDA**

Photo 1: Evolution of mycelial growth according to the different concentrations of essential oil after 7 days of incubation.

4. Discussion

The presented results are in agreement with those of Merghache *et al*. (2009) who showed that the yield of essential oil of *R. chalepensis* collected from Tlemcen (West Algeria) and obtained by hydrodistillation has a yield varies according to the place and period of harvest, respectively of (0.35-1.28)% and (0.28-0.84)%. While the yield of essential oil is lower than those mentioned in the work of Aouadhi *et al*. (2013) and Daoudi *et al*. (2016) which obtained a yield, respectively 0.85%; and 1%. Our study showed that the composition of *R. chalepensis* essential oil is rich in oxygenated monoterpens, especially camphor (76.57%). The GC/MS analysis of extracted essential oil of *R. chalepensis* from Beni Mester (West Algeria) revealed 20 compounds representing 64.66-93.99% of total oil. The major component was 2-undecanone (20.40 - 82.74%) **(**Merghache *et al*., 2009). Indeed, other studies have indicated that the essential oil extracted from *R. chalepensis* from Grarem (East Algeria) is characterized by the abundance of 2-acetoxytetradecane (58.44%), 2-acetoxytridecane (19.07%) and 2-tridecanone (6.39%) (Chibani *et al*., 2013). However, the works of Ben Bnina *et al*. (2010), Mejri *et al*. (2013) and Majdoub *et al*. (2014) on the same plant originally from different Tunisian regions reported two major constituents: nonan-2-one and undecan-2-one. While the essential oil of *R. chalepensi* from Kef (Tunisia) has several major constituents: menthol (49.92%), linalool (31.1%) and 2-hexanal (5.2%) (Aouadhi *et al*., 2013). Variation in yield and chemical composition of essential oil of *R. chalepensis* may be related to several factors, such as: geographical location, duration and place of drying, temperature, technique and time of extraction, soil type, environmental conditions, diseases caused by exogenous agents, techniques of plant harvesting and extraction, the vegetative plant stage (Daoudi *et al.*, 2016; Bergheul, 2018; Atailia and Djahoudi, 2015).

In this study, the essential oil of *R. chalepensis* characterized by the relatively high content of camphor exhibited an important antifungal power on the tested fungus. These findings suggest that this strong antifungal effect could be related to these major components. Indeed, other reports showed that oxygenated monoterpens as active antimicrobial agents (Lucini *et al*., 2003; Kordali *et al*., 2003).

Aouadhi *et al*. (2013), Ben Bnina *et al*. (2010) and Bergheul (2018) have demonstrated that the essential oil of *R. chalepensis* from Algeria had significant antifungal activities against *Aspergillus niger*, *Aspergillus flavus*, *Alternaria* sp, *Trichderma* sp, *Fusarium oxysporum*, and *Botrytis cinerea* and *Candida albicans*. Indeed, Kordali *et al*. (2003) demonstrated that oxygenated monoterpens compounds of *Artemisia santonicum* and *Artemisia spicigera* essential oils have higher antifungal activities. Wali e*t al*. (2019) have shown that plants can manufacture diverse bioactive metabolites, and those metabolites have the potential for huge pharmacological action. Mathe (2018) has demonstrated that the use of medicinal and aromatic plants in plant protection, especially the versatile forms of utilization of essential oils is promising. The antifungal activity of essential oil can be explained by the synergistic effect between the different essential oil compounds. Lucini *et al*. (2006) showed that sclerotial differentiation was delayed mainly by camphor, 1.8-cineole, linalool and menthol. Sharma and Tripathi (2006) exhibited that essential oils caused degeneration of fungal hyphae that appeared empty of their cytoplasm content, and loss of rigidity and cell wall integrity.

5. Conclusion

In conclusion, This study consisted in evaluating the antifungal capacity of the essential oil of *R. chalepensis* collected from M'chounech (Wilaya of Biskra) against the mycelial growth of *M. scaettae*, fungus responsible for the inflorescence rot of date palm (*Phoenix dactylifera* L.). The results of this work demonstrated that essential oil could be used as a potential antifungal agent. The chemical analysis of the different compounds of *R. chalepensis* essential oil showed the presence of oxygenated monoterpens. A significant effect was observed against the studied fungus strain. The essential oil tested allowed to limit considerably the development of *M. scaettae* compared to the control. Finally, this study can be exploited for the use of *R. chalepensis* essential oil as a new control strategy against this major constraint of date palm.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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