Original Research Article

Antifungal activity of essential oil from Tunisian myrtle (*Myrtus communis* L.)

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Novelty statement: This paper highlights the antifungal activity of myrtle (*Myrtus communis* L.) essential oil. Myrtle essential oil was used on seven fungi, *Fusarium oxysporum f.sp. Lycopersi, Fusarium solani, Sclerotium rolfsii, Sclerotinia sclerotiorum, Aspergillus niger, Aspergillus flavus* and *Penicillium digitatium*. Results confirmed the effectiveness of this oil to be utilized as a biological fungicide. The efficient antifungal activity of myrtle essential oil comes from its high content in mono-terpenic alcohols and mono-terpenic hydrocarbons.

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Abstract

The objective of this study was to investigate the antifungal essential oil activity. The *InVitro* antifungal activity of myrtle essential oil against seven fungi allowed us to specify the corresponding minimum inhibition concentration (MIC) of mycelial growth for each fungal strain. Results showed evidence for a strong inhibitory activity of this essential oil. Concentrations of 5 µl/ml and 9 µl/ml were sufficient for the total inhibition of the development of *S. rolfsii* and *S. sclerotiorum*, respectively. When used on potato tubers by applying the MIC 100% of *S. rolfsii* or *F. solani*, the fungicidal effect varied according to the type. Mean penetration of *S. rolfsii* was significantly inhibited (3 mm) by myrtle essential oil used as a preventive control in comparison with the inoculated control (12 mm). Myrtle oil was able to control the white rot of potato tubers caused by *F. solani* by reducing the average penetration by 70%. Myrtle essential oil have promising antifungal effects to be valorized in agricultural activities.

Keywords: Mediterranean shrubs; biological control; fungi; minimum inhibition concentration; mycelial growth.

Introduction

Mediterranean shrubs including myrtle; have been traditionally used as foods, flavors, spices and plant protection products (Sanjust et *al*., 2008). Myrtle (*Myrtus communis* L.) is an evergreen shrub belonging to the Myrtaceae family. It develops spontaneously in Tunisia. It generally grows on siliceous soil and in humid to semi-

humid ecosystems (Benabid, 1997). The benefits of myrtle are diverse, the different parts of the plant are used in cosmetology and medicine, and the essential oil is used in the composition of liqueurs, perfumes, beauty care and aromatherapy. The leaves, fruits, flowers and roots are indicated as a remedy for several diseases (Chalchat et *al*., 1998; Elfellah et *al*., 1984). The main objective of this study was to evaluate the biological activities of antifungal essential oil of the Tunisian Myrtle.

2. Materials and methods

2.1. Biological material

The essential oil is obtained by the hydro-distillation of the leafy branches of myrtle (Figure 1). The plant materials used for the extraction of essential oils were harvested in January 2015 from mature shrubs found in the region of Ghardimaou, governorate of Jendouba in the Northwest of Tunisia (Figure 2).

Figure 1. Leafy branches of myrtle (*Myrtus communis* L.).

Figure 2. Location of the Ghardimaou study area.

2.2. Essential oil analysis

The essential oils' composition was assessed using Gas chromatography–mass spectrometry (GC/MS) analysis. This analysis was performed on an HP 5972 mass spectrometer (Agilent technologies, Palo Alto, California, USA) with electron impact ionization (70 eV). An HP-5MS capillary column (30 m-0.25 mm coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, and 0.25 lm film thickness) were used. The oven temperature was programmed to rise from 50 to 240 °C at a rate of 5°C/min; transfer line temperature was 250°C. The carrier gas was He with a flow rate of 1.2 ml/min and a split ratio of 60:1. Scan time and mass range were 1s and 40–300 m/z, respectively. The identification of volatile compounds was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC/MS data system and other published mass spectra (Adams, 2001). The determination of the percentage composition was based on peak area normalization without using correction factors.

2.3. Fungi material

The antifungal activity of the myrtle essential oil was studied against several pathogenic fungi. Different fungal isolates of *Penicillium digitatum, Aspergillus niger,* Aspergillus flavus, *Fusarium solani, Fusarium oxysporum* f. Sp. *Lycopersici, Sclerotium rolfsii* and *Sclerotinia sclerotiorum* were provided by the Phytopathology Laboratory of the Chott Mariem Regional Center for Research in Horticulture and Organic Agriculture. The medium used for the cultivation of fungi was the PDA (Potato Dextrose Agar).

2.4. In vitro antifungal activity of myrtle essential oil

2.4.1. Direct contact technique

Direct contact activity was studied by applying the poisoned food technique adopted by Grover and Moore (1962). This technique consists of adding the essential oil at different concentrations (between 0.5 and 10 µl / ml) to the culture medium (PDA) in addition to a control. The inoculation was carried out by depositing the agar disks of the mycelium 6 mm in diameter. The percent inhibition of mycelial growth was determined according to the formula of Pandey *et al*. (1982).

Percentage inhibition = ((dc - dt) / dc) × 100

dc: Diameter of the mycelial colony of the untreated control.

dt: Diameter of the mycelial colony treated with essential oil.

2.4.2. Study of the effect of essential oil vapor

The minimum inhibitory concentrations (MIC) for the different fungi strains were used to determine the inhibition rate of Myrtle essential oil tested by the essential oil vapor effect method. To study this effect, the micro-atmosphere test was adopted (Mahanta et *al*., 2007). Indeed, in a PDA medium, mycelium agar disks were placed

in each box and above them a disk of filter paper soaked in the essential oil at the minimum concentration of inhibition relative to each fungus. Mycelial growth was determined daily for each treatment.

2.5. In vivo antifungal activity of myrtle essential oil

The treatment of the potato tubers with the essential oil consists of applying their total inhibition concentration determined during in vitro tests against *S. rolfsii* and *F. solani strains*. The technique for inoculating potato tubers is that adopted by Tivoli and Jouan (1981) and Daami-Remadi et *al*. (2011). In fact, the tubers are injured in their middle part by means of a punch. The wound is 6 mm in diameter and depth. An agar plate carrying the pathogen originating from a 9-day culture incubated at 25 °C was then deposited in this inoculation site before and after their treatment with the essential oil of myrtle.

The average penetration was computed using a formula proposed by Lapwood et *al*. (1984) integrating the width and depth of the rot caused:

$P = (L/2 + (p-6)) / 2$

With P: Mean penetration (mm); L: Width (mm) and p: Depth (mm).

2.6. Statistical Analysis

The analysis of the variance was performed by the ANOVA method. The comparison of means from three replicates was done by the Student-Newman-Keuls test using the SPSS 20 software with 0.05 being the default significance level for rejection of a null hypothesis $\mu_i = \mu_j$. All data was expressed as mean ± standard error. Minimum concentrations of 80% inhibition were estimated following a probit analysis using the method of Finey (1977).

3. Results

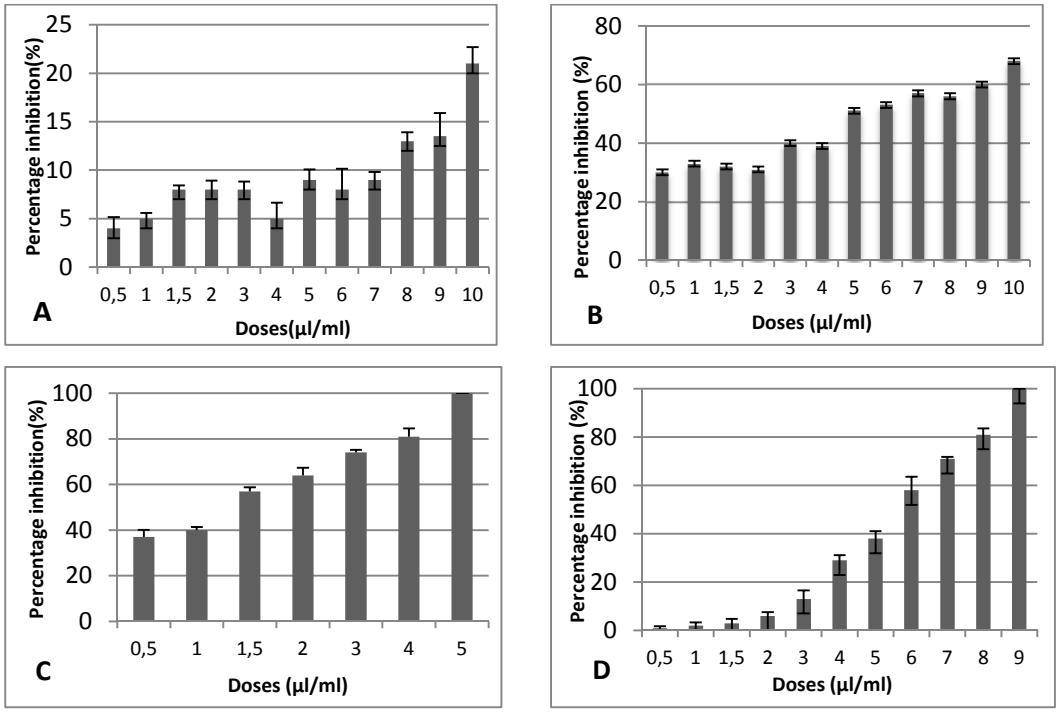
The Myrtle essential oil compounds are given in Table 1. The chemotype in *Myrtus communis* is α-pinene (29.84 %) a characteristic of the Tunisian Myrtle (Zaouali et *al*. (2008), Yadegarinia et *al*. (2006); Chalchat et *al*. (1998)). However, the Myrtle essential oil content in the main components are different from other results reported in Tunisia, Morocco and Greece. The level of limonene and linalool are higher than our results depending on the 1-8 cineol level (Snoussi et *al*., 2008; Viuda-Martos et *al*., 2007; Abidi-wannes et *al*., 2010).

Table 1. Main compounds of the myrtle essential oil

α-pinene (29.84 % ±1.03)
Limonene (20.59 % ±0.85)
Linalol (11.45 % ±0.64)
linalyl acetate (10.00 % ±0.75)
geranyl acetate (5.88 % ±0.35)
1.8 cineole (2.29 % ±0.24)
p-cymene (1.49 % ±0.05)
α-humulene (1.58 % ±0.08)
α-terpineol (1.35 % ±0.09)
neryl acetate (1.09 % ±0.12)

The study of the antifungal activity of myrtle oil was reflected by the percentage of inhibition of mycelial growth of each fungal species relative to the untreated control. A significant interaction was observed between the fungus and the different concentrations tested (Figure 3). The fungicidal activity of the different fungal strains versus myrtle oil is different from one strain to another. The fungicidal activity for *A. niger* (Figure 3A) was too low and did not exceed 21.15% even for high

doses ranging from 8 to 10 µl/ml. For *A. flavus* (Figure 3B), the results were more interesting since it was possible to have a percentage of inhibition in the order of 68% at 10 µl/ml of concentration. Although in the case of *S. rolfsii* (Figure 3C), the concentration of 5 µl/ml was sufficient for the total inhibition of its development with a significant difference between most of the doses tested. In the case of *S. sclerotiorum* (Figure 3D), the concentration of 9 µl/ml was sufficient for the total inhibition of its development. In fact, at 10 µl/ml, *F. solani* (Figure 3E) has reached an inhibition level in the order of 32%. *F. oxysporum* (Figure 3F) was inhibited to a maximum of only 34% at 10 µl/ml. Finally, for *P. digitatum* (Figure 3G), the maximum inhibition reached was 33% at 7 µl/ml.



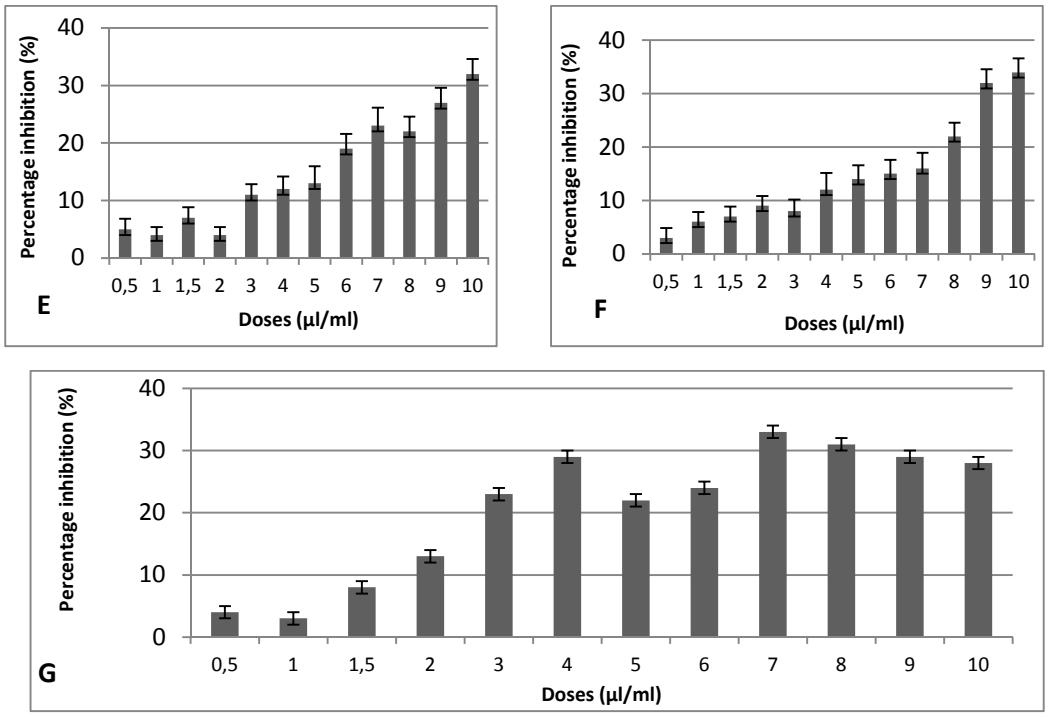


Figure 3. Inhibition rate of mycelial growth of fungi (*A. niger* (A), *A. flavus* (B), *S. rolfsii* (C), *S. sclerotiorum* (D), *F. solani* (E), *F. oxysporum* (F), *P. digitatum* (G) subjected to treatment with myrtle essential oil after 6 days incubation at 25 °C ($p \leq 0.05$).

The growth of *S. rolfsii* was inhibited relative to the control for three days of incubation but at a rate of inhibition that decreased over time. Indeed, this percentage was 58.45% after one day, 28.83% after two days and 11.84% after the 3rd day. In the case of *S. scleroetiorum*, the inhibition was 25.2% after two days of incubation and 10.78% after three days (Figure 4). The treatment of potato tubers with myrtle essential oil consists in applying their total inhibition concentration determined during our in vitro tests against *S. rolfsii* and *F. solani*.

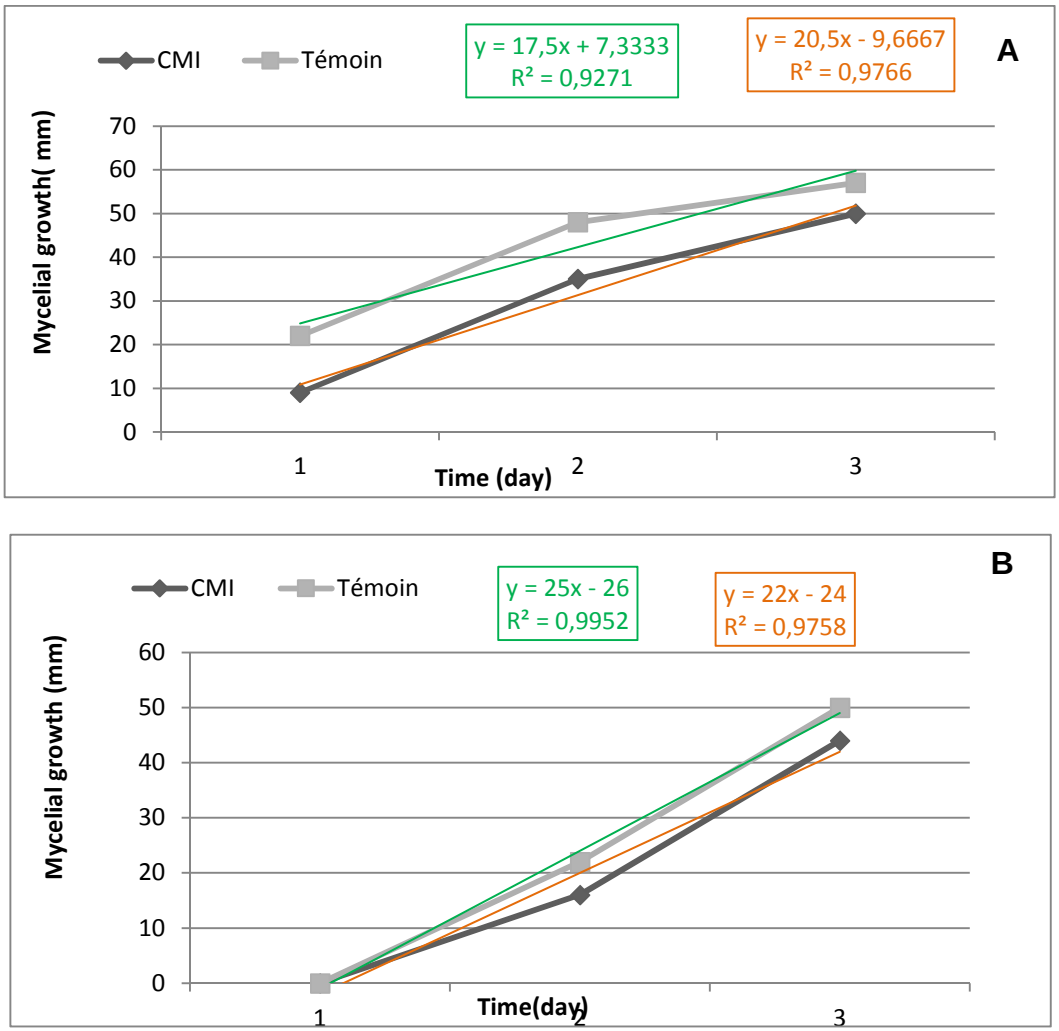


Figure 4. Fungistatic activity of the myrtle essential oil on the mycelial growth of *S. rolfsii* (A) and *S. sclerotiorum* (B) at 25 ° C. of incubation.

The use of essential oil as a means of preventive control consists in applying the lethal doses before inoculation of potatoes by the two fungi responsible for tuber rot. The results obtained show that the average penetration of *S. rolfsii* was significantly inhibited by our essential oil tested in comparison with the inoculated control. This result highlights the preventive action of this oil to protect tubers from the infection by this fungus. While the essential oil of myrtle has a very important effect against this

disease with a maximum percentage of 76.32%. Indeed, myrtle oil was able to control the white rot of the potato tubers by reducing the average penetration by 70% *F. solani*.

4. Discussion

According to our results, we can observe that the myrtle essential oil studied showed antifungal activities varying from one fungus to another and depending on the concentration of the oil. It seems to be effective as a biofungicide since it has given reliable results especially against *S. sclerotiorum* and *S. rolfsi* with a rate of inhibition of mycelial growth which reaches 100%. According to Vokou et *al*. (1988), the fungistatic activity of aromatic compounds of an essential oil appears to be related to the presence of certain chemical compounds. Then, the biological activity of an essential oil must be related to its chemical composition (alcohols, phenols, terpene compounds and ketones) and the possible synergistic effects between these components. Thus, the nature of the chemical structures that constitute it and also their proportions play a decisive role in its fungistatic activity (Dorman and Deans, 2000). For in vivo tests on potato tubers, the results show that the fungicidal effect varies depending on the type of fungus. Indeed, for *F. solani*, the total MIC by the essential oil of myrtle is effective to inhibit the dry rot following their use as a means of curative control. Thus, for *S. rolfsii*, one notes that the use of the essential oil of myrtle has shown good results as a means of preventive control.

The sensitivity of strains to essential oil can only be explained by the richness of the chemical composition of this essential oil (Aberchane *et al.*, 2003). Also, anti-bacterial and anti-fungal activity differs from one plant to another even though they belong to the same family (El Ouali Lalami *et al.*, 2013). It has been established that the bactericidal and fungicidal action of alcohols in essential oils is superior to that of

ketones and hydrocarbons (El Bouzidia *et al.*, 2013). Knowing that our essential oil includes: α -pinene (29.84%), limonene (20.59%) and p-cymene (1.49%) were mono-terpenic hydrocarbons with marked anti-infectious action; Linalol (11.45%) and α-terpineol (1.35%) were mono-terpenic alcohols with more potent antifungal properties.

The main constituents of myrtle essential oil responsible for antifungal capacity in vitro and in vivo are Linalol, α-terpineol, α -pinene, limonene and p-cymene. This activity is the result of a majority component or a synergy between several components (Hennia et *al*., 2015, Bahmanzadegan et *al*., 2013).

Conclusion

The purpose of this work is to study the biological activities of antifungal essential oil of aromatic and medicinal plants such as myrtle against *A. niger*, *A. flavus*, *S. rolfsii*, *S. sclerotiorum*, *P. digitatum*, *F. solani* and *F. oxysporum* f.sp. *Lycopersi* by the method of dilution in vitro agar medium as well as potato tubers in vivo. The results obtained for the in vivo tests are very interesting in order to approximate the natural conditions. They have shown the effectiveness of using these essential oils as a means of preventive control. In view of these results it would be advantageous to use these natural products which are biodegradable by proceeding with a preparation for treating fruits in the form of emulsions or for treating storage rooms in the form of fumigants or solutions for the preparation of baths where we could soak fruit before preserving. As a result, this control method can be used as an alternative to biological control as part of an integrated post-harvest disease control strategy.

Acknowledgments

This work was supported by the Institution of Agricultural Research and Higher Education, the International Center for Agricultural research in the Dry Areas (ICARDA) and the Arab fund for Social and Economic Development (AFSED).

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