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Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) and its *in vitro* inhibitory effect on *Penicillium digitatum*

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Abstract

The objective of this study was to evaluate rosemary (*Rosmarinus officinalis* L.) growing wild in Hammam Dalâa (Algeria) for total phenolic content, free radical scavenging activity (FRSA) and test the effect of its extracts on the growth of the green mold of citrus, *Penicillium digitatum*, under *in vitro* conditions. The results obtained using the Folin-Ciocalteu method showed a high content of the extract in polyphenols reaching 129 mg gallic acid equivalents/g and relatively low content of flavonoids (38mg of quercetin equivalents/g dry extract). In the DPPH assay the methanol extract exhibited a FRSA close to those of the synthetic antioxidants, tested as positive controls, and higher compared to that of the EO. The *in vitro* antifungal assay by means of different methods showed a clear inhibitory effect of the rosemary essential oil and methanol extract on *P. digitatum*. The inhibitory effect registered at the 6th day of incubation at 25°C was between 5 to 79% when the essential oil was applied as fumigant at concentrations ranging from 10 to 50 µl, and was between 13 and 50% when applied by contact bioassay at levels from 1000 to 3500 µl/l. Spore germination was strongly affected by the oil applied by disk diffusion method. The methanol extract exhibited inhibition exceeding 50% of the fungus mycelial growth but at relatively high concentration 0.8g/l. These results support the studies on rosemary as a promising source of preservatives.

Introduction

Citrus is an important fruit crop grown commercially in more than 135 countries in different agro-climatic conditions and its postharvest diseases result in serious economic damage (Naqvi, 2004). Injuries on citrus fruit caused during harvest, handling, transport, storage and marketing, provide entries to wound pathogens, including *Penicillium digitatum* Sacc. and *P. italicum* Wehmer, causal agents of green and blue mould, respectively (Zamani *et al.*,2009).

Citrus fruits are among the major perennial crops in Algeria. They represent 27% of the total fruit production and occupy an area of 11% compared to other fruit crops. Seventy nine percent of production is destined for fresh consumption, processing is around 8.000 t/year. The citrus processing industry concerns concentrates, natural juices and jams (Kerboua, 2002; Ghezli and Aouane, 2002; Biche, 2012). Disease losses caused by fungal wound pathogens in Algerian citrus fruits are not well documented.

P. digitatum grows on the surface of postharvested citrus fruits producing characteristic powdery olive-colored conidia and is commonly known as green mold. This pathogen is of main concern as it is responsible for 90% of citrus losses due to diseases occurring during the storage period, and it causes serious damages in commerce (Ariza *et al*., 2002). P. digitatum repeated cycles of infection and sporulation commonly occur in packed fruits. It can also cause an allergic response due to the enormous numbers of dry air-borne spores it produces (Mekbib *et al*., 2011).

In spite of fungicide use and the increased implementation of new alternative strategies, green mold in citrus continue to represent a major problem for stored fruits worldwide (Buron-Moles *et al.*, 2012). Moreover, and due to the development of resistance to fungicides (Viñas *et al*., 1993), as well as concerns about the environment and public health, the use of synthetic fungicides is becoming increasingly restricted (Palou *et al.*, 2001).

Due to the demands of the consumer market for food without the presence of pesticide residues, there is a growing interest in the development of safer, effective alternative compounds to control postharvest fungal diseases (Mekbib *et al.*, 2011). The exploitation of natural products, such as essential oils and plant extracts, to control decay

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and prolong storage life of perishables has received more and more attention (Tripathi and Dubey, 2004) and recently gained a great popularity and scientific interest (Nuzhat and Vidyasagar, 2013).

Some aromatic plants with known antiseptic and antimicrobial properties and mainly used as spices in food preparations, have been studied for use in the control of moulds (Centeno *et al*., 2010). *Rosmarinus officinalis* is one of the oldest known medicinal plants in Algeria; it has several uses of which as antispasmodic and as a flavor and fragrance ingredient in food (Boutekedjiret *et al.*, 1998; 2003; Djeddi *et al*., 2007, Hendel *et al*., 2012)

The aim of our work was to evaluate rosemary methanol extract (ME) and essential oil (EO) for total phenolics and antioxidant activity, and to investigate their effect on *P. digitatum* isolated from oranges commercialized in local food markets.

Materials and Methods

Plant material

The plant material used for this work is Rosemary (*Rosmarinus officinalis* L.) growing wild in the region of Hammam-Dalâa (M'sila). Collected during the month of March 2014, fresh material (aerial parts: leaves and flowers) was dried under shade at room temperature and leaves and flowers were removed then stored in clean paper bags until use.

Essential oil extraction

The prepared air-dried plant material of rosemary was powdered and 100 g were submitted to hydrodistillation for 3 h with 1000ml distilled water using a Clevenger-type apparatus. The extracted oil was collected and dried over anhydrous sodium sulfate, then stored in sealed glass vials in a refrigerator at 4˚C until use. The yield based on dry weight of the sample was calculated.

Preparation of the methanol extract

The procedure was done as described by Erkan *et al.* (2008) with some modification. Thirty grams of the powdered plant material were subjected to Soxhlet extraction using 300 ml of methanol as solvent at 40°C for 8 hours. Methanol containing the extract was then filtered through Whatman paper. The methanol extract was concentrated under reduced pressure on a rotary evaporator. Extract was kept in dark at 4˚C until use. The procedure was repeated three times.

Determination of the total phenolic content

The content of total polyphenols was quantified in the methanol extract by the Folin-Ciocalteu spectrophotometric method using gallic acid as standard according to the procedure described by Wong *et al.* (2006) with slight modifications. Briefly, 100 µl of the extract was mixed with 2.5ml of Folin–Ciocalteu reagent (10x dilutions). After 5min reaction, 2.5 ml of Na_2CO_3 solution (7.5% w/v) was added and allowed to stand for 2 h. The absorbance was measured at 765 nm in a spectrophotometer. The concentration of total phenolic compounds was expressed as mg gallic acid equivalents (GAE) per g dry extract by using an equation obtained from standard gallic acid graph.

Determination of the total flavonoid content

The flavonoids content in the extract was estimated spectrophotometrically according to Khlifi *et al*. (2013). Briefly, a diluted solution (4 ml) of the extract was mixed with an equal volume of aluminium trichloride ($AlCl_3$) in methanol (2%). The absorbance was read at 415 nm after 15 min against a blank sample consisting of a methanol (4 ml) and extract (4 ml) without $AlCl_3$. Quercetin was used as reference compound to produce the standard curve, and results were expressed as mg of quercetin equivalents (QE) per g of dry mass.

DPPH assay

The DPPH assay was carried out as described elsewhere (Tepe *et al*., 2004; Hatipoglu *et al.*, 2013). Fifty microliters of various concentrations of the extract or the essential oil were added to 5ml of a 0.004% (w/v) methanol solution of DPPH. After a 30min incubation period at room temperature the absorbance was read against a blank at 517 nm. The inhibitions of DPPH radical in percent (I%) were calculated as follows:

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

where A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance value of the extract or the essential oil. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract or oil concentration. Tests were carried out in triplicate and Butylated HydroxyAnisole (BHA), butylated hydroxytoluene (BHT) and ascorbic acid (AA) were used as positive control.

Fungal strain used in in vitro *antifungal assay*

P. digitatum strain used in this study was isolated from decayed oranges. The identification of the

fungus was performed according to the macro and micro-morphological characteristics described by Pitt and Hocking (2009). The fungal strain was maintained on Potato Dextrose Agar (PDA) plates and slants at 5°C, with periodic transfers through orange fruit to maintain its aggressiveness.

Preparation of the conidial suspension

Conidial suspension was prepared as described by Nguefack *et al.* (2009): conidia were harvested from 10-day-old culture by pouring a sterile 0.01% aqueous solution of Tween 80 onto the culture plates and scraping the plate surface with a sterile bent glass rod to facilitate the release of conidia. Using a Heamocytometer, the number of conidia in the suspension was adjusted to approximately 10^6 conidia ml^{-1}.

Effect of rosemary on the growth of the fungus

The effect of rosemary, essential oil and extract, on the fungus was conducted using different methods.

Contact bioassay

The experiments were conducted according to the method of Marandi *et al.* (2011). PDA medium was autoclaved and cooled in a water bath to approximately 45°C. The tested oil was aseptically added at different concentrations: 1000, 1500, 2000, 2500, 3000, and 3500 µl/l to the molten PDA containing Tween 80 (0.5% v/v). The resulting media were immediately dispensed (15 ml) into sterilized Petri plates (9 cm) then inoculated at the center with 6mm plugs from the fungal 7-days-old cultures. In the control, water was used instead of the essential oil. Inoculated Petri plates were incubated at 25°C in darkness and observations were recorded daily up to the 6th day. Three replicates were used per treatment. Experiments were performed twice. Mycelial growth inhibition (MGI %) was calculated by the followed formula:

$$MGI\ (\%) = ((dc-dt)/dc) \times 100$$

where *dc* and *dt* represent mycelial growth diameter in control and treatment Petri plates, respectively.

Fumigation bioassay

The Fumigation bioassay was carried out as described elsewhere (Neri *et al*., 2006; Feng *et al*., 2011). A mycelial disc (6 mm diameter) was taken from the periphery of an actively growing agar culture (7-days-old) and placed at the center of a 90 mm Petri dish containing 15 ml of PDA. Different concentrations volumes (10, 15, 20, 30, 40, and 50 µl/plate) of the essential oil were added on sterilized filter paper disc (9 mm diameter) placed on the cover inside the dish kept in an inverted position. Three replicates were used per treatment. Experiments were performed twice. The dishes were quickly sealed with parafilm and incubated at 25°C. Control treatments consisted of Petri dishes inoculated with the fungus but treated with distilled water instead of the essential oil. The fungi growth recorded daily up to the 6th day. Growth inhibition was calculated as the percentage of the inhibition of radial growth relative to the control.

Disc-diffusion assay

Aliquots (100 µl) of the fungal spore suspension (10^6 spore ml^{-1}) were spread on PDA medium. The paper discs (6 mm diameter) individually impregnated with 15, 20 and 25 µl of essential oil were placed on the inoculated agar. Inoculated plates were kept in the refrigerator (4°C) for 2 h and then incubated at 25°C for 72 h. Distilled water was used as negative control. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. The effect was calculated as a mean of triplicate tests. Each assay in this experiment was repeated twice (Tepe *et al*., 2005; Gulluce *et al*., 2007; Zaouali *et al*., 2010; Hosni *et al*., 2013).

Agar-well diffusion method

The methanol extract was screened for its antifungal activity using the well-plate diffusion method (Talibi *et al*., 2012). Wells (6-mm-diameter) were made at three locations per Petri plate containing 20 ml of PDA. The wells are then filled with 10 and 20 µl of solvent extract at concentration of 0.8 g ml^{-1}. Control plates consisted of wells filled with the solvent Dimethyl Sulfoxide (DMSO). A mycelial disc (6mm diameter) was taken from the periphery of an actively growing agar culture (7-days-old) and placed at the center of the dish containing the extract. Inoculated Petri plates were incubated at 25°C in darkness and observations were recorded daily up to the 5th day. Experiment was performed twice. Mycelial growth inhibition (MGI %) was calculated by the above formula.

Statistical analysis

All experiments were conducted in triplicate and data expressed as mean ± SD. The analysis of variance (ANOVA) and Tukey's multiple comparison were considered significant at $p<0.05$. The results obtained in the well-plate diffusion method were statistically analyzed by means of Student t-test (All the statistical analyses were accomplished using the computer software GraphPad prism 6.05 for

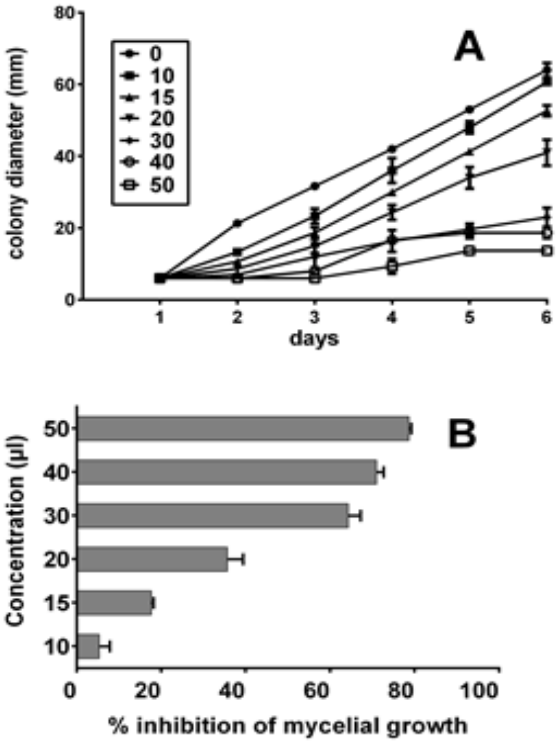

Figure 1. The growth of *P. digitatum* on PDA under vapored rosemary essential oil (µl) (A), and the inhibitory effect of the oil on mycelial growth at the 6th day (B). Data are averages ± SD and differences among means are statistically significant (P<0.05) according to Tukey's multiple comparisons test

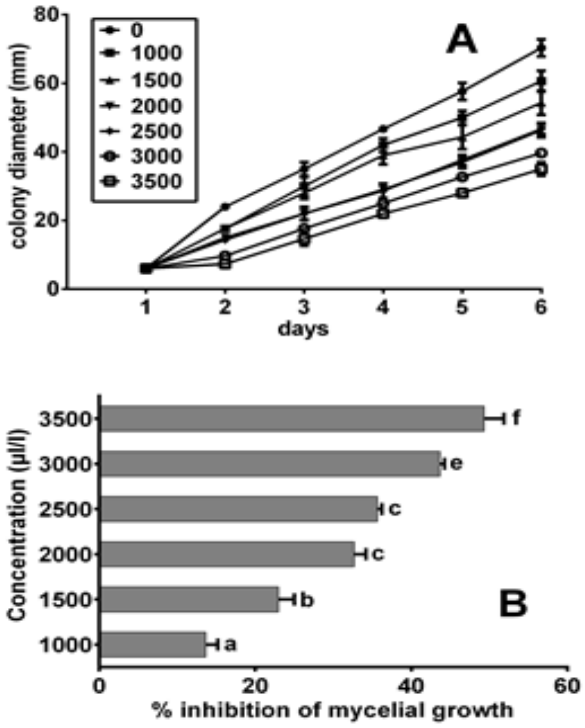

Figure 2. The growth of *P. digitatum* on PDA with incorporated rosemary essential oil (µl/l) (A), and the inhibitory effect of the oil on mycelial growth at the 6th day (B). Data are averages ± SD and different letters indicate significant differences (P<0.05) according to Tukey's multiple comparisons test

Microsoft Windows).

Results and Discussion

Yields of the plant extracts

The plant EO yield was 1.15 ± 0.102% (v/w). Studies have shown variations in yields of rosemary essential oil depending on the plant variety and the ecological characteristics of the collection area (Jamshidi *et al*., 2009; Zaouali *et al*., 2010; Ojeda-Sana *et al*., 2013). Yield will vary if the plant is cultivated or spontaneous (Atik Bekkara *et al*., 2007), and according to the method of extraction (Bousbia *et al.*, 2009). Fresh and dry aerial parts harvested in March and April in an Algerian subhumid zones; Algiers and El Kala, gave yields of 0.82% and 0.36% respectively (Djeddi *et al*., 2007; Ouibrahim *et al*., 2013). Rosemary EO yields of neighboring regions of our collection site (Bibans in the region of Bordj Bou Arreridj) vary between 0.44 and 1.5% (v/w) (Boutekedjiret *et al*.,1998; 2003). At an Algerian western area (Tlemcen) the yield is around 1.2% (Haddouchi *et al.*, 2009). According to the applied procedure, methanolic extraction yielded in 31.867 ± 3.395g.

Determination of the total phenolic and flavonoid content

The total phenolic content of the plant methanol extract, determined by the Folin-Ciocalteu method, was found to be 128.976 ± 9.257mg of GAE per g dry extract. Flavonoids were lower and were estimated to be 38.018 ± 0.884mg of QE per g dry extract. These results indicate the richness of the tested plant in polyphenols. This is clear when compared to studies using Folin-Ciocalteu method on Turkish rosemary reporting levels varying from 147.3 to 34.1mg GAE per g extract, regardless of collection time (Yesil-Celiktas *et al*., 2007b), and the cultivated plant in Argentina (Moreno *et al.*, 2006). Others have shown lower phenolic contents of the rosemary extract (Moreno *et al*., 2006; Wojdyło *et al.*, 2007; Santos *et al*., 2012).

DPPH assay

The hydrogen atom or electron donation ability of the EO or MeOH extract was measured from the bleaching of purple-coloured methanol solution of DPPH. This stable free radical is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods (Esmaeili and Sonboli, 2010). The DPPH FRSA depends on the ability

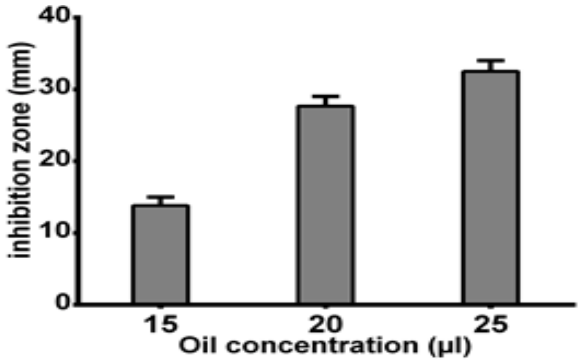

Figure 3. Mean diameter (mm) of inhibition zones of *P. digitatum* growing on PDA medium containing impregnated paper discs of rosemary oil. Data are averages ± SD and differences among means are statistically significant (P<0.05)

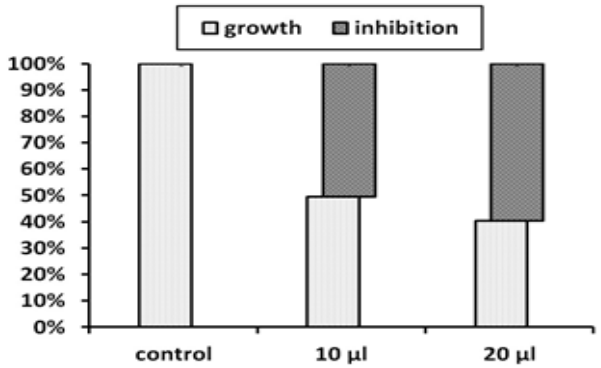

Figure 4. The growth of *P. digitatum* on PDA plate under methanol extract effect using the well-plate diffusion method. Data are averages ± SD and differences among means are statistically significant (P<0.05)

of DPPH to be decolorized in the presence of antioxidants (Sourov *et al*., 2014). The IC_{50} values (the concentration with scavenging activity of 50%) showed that the tested rosemary ME exhibited a high radical scavenging activity (11.741± 0.004µg/ml) close to those of the tested synthetic antioxidants AA (3.036± 0.217µg/ml), BHA (7.492±0.057µg/ml) and BHT (21.211± 2.593µg/ml), but higher compared to that of the EO (3.53±0.038µl/ml). Compared to other studies (Okoh *et al.*, 2011; Miladi *et al.*, 2013), our tested rosemary exhibited a high FRSA. Ojeda-Sana *et al*. (2013) reported a high scavenging capacity related to a chemotype of rosemary essential oil rich in myrcene, but Wang *et al.* (2008) and Hussain *et al.* (2013) mentioned a greater activity of *R. officinalis* essential oil better than its main components, namely, 1,8-cineol, camphor, a-pinene; the major compounds and also minor compounds may make a significant contribution to the oil's activity. From the chemical point of view about plant essential oils, monoterpene hydrocarbons and oxygenated monoterpenes have the main role in the protective action followed by sesquiterpene hydrocarbons (Ruberto and Baratta, 2000). Yesil-Celiktas *et al.* (2007) pointed out that the composition of the rosemary extract change according to the type of the sample, the location and time of harvest thereby various extracts from different geographical locations and from different time-points also varied significantly. The antioxidant activities in rosemary extracts could be due to carnosol, carnosic acid, rosmarinic acid, rosmanol, and rosemaridiphenol phenolic compounds (Rababah *et al*., 2004). The physicochemical nature of the individual phenolics in the extracts may be more important in contributing to the antioxidant activity than the total phenolic content (Santos *et al*., 2012).

In vitro *antifungal assay*

Generally all concentrations applied by the different methods, reduced the growth of the tested fungus. Some significantly reduced or completely inhibited the growth. No sporulation was observed. For fumigation bioassay, concentrations 30, 40 and 50µl inhibited growth with a rate higher than 50% throughout the incubation period with complete growth inhibition at the 5th day for 40 and 50µl (Figure 1A). Low abundance of growing mycelium and no sporulation was observed. On the 6th day inhibition was estimated at 5 to 79% (Figure 1B).

Compared to the fumigation bioassay, concentrations tested by contact bioassay method showed less activity (Figure 2A); the fungus kept the same rate of growth with all tested concentrations and inhibition at the 6th day was estimated to be between 13 and 50% depending on the tested oil concentration (Figure 2B). In the disk diffusion assay, the effect was very strong on spore germination and the diameter of the inhibition zone was estimated 14, 20 and 32.5mm at the concentrations 15, 20 and 25µl respectively, with lack of sporulation and sparse mycelium compared to the control (Figure 3). The well-plate diffusion method concerned only the methanol extract at concentration 0.8g/ml. This relatively high concentration exhibited inhibition higher than 50% compared to the control test (Figure 4).

Many plants, particularly those belonging to the Lamiaceae family are known for their antimicrobial activities and especially their EOs. The different methods used allowed us to know that rosemary has significant antifungal activity and especially its EO. This presented a significant inhibitory effect compared to the extract. These findings are in agreement with Yigit *et al*. (2000) who reported that vapor effect of rosemary oil prevented mycelial colour and inhibited growth as 71.4% and reduced decay by 12.5% on the

treated orange fruits with 900 ppm. Some previous research on rosemary cited the antifungal activity of its EO evaluated as average against *P. digitatum* and other fungal species, compared with that of thyme EO estimated very active (Benjilali *et al*., 1986). Nevertheless, Marandi *et al*. (2011) showed that the rosemary EO showed an inhibition percentage greater than *Thymus kotschyanus* and *Ocimum basilicum* when tested on *P. digitatum*. Other studies reported the high sensitivity of *P. digitatum* to the plant EOs and extracts according to the type of solvent (Ameziane *et al.,* 2007). Yesil-Celiktas *et al.* (2007a) reported the effectiveness of rosemary EO as methanol extract against *Candida albicans*. These antimicrobial activities are due to the chemical composition of the EOs and leaves which contain important compounds namely α-pinene, bornyl acetate, camphor, 1,8cineol and rosmarinic acid (Kocie-Tanackov and Dimie, 2013).

Plants extracts and EOs have proven their *in vitro* effective action on a large variety of fungi (Apisariyakul *et al*., 1995; Zabka *et al*., 2009; Prakash *et al.*, 2012; Askarne *et al*., 2013). The mode of action of essential oils on *P. digitatum* and other fungi has not been determined and it has been shown that their antimicrobial activity is dependent on their hydrophobicity and partition in microbial membranes. In general antimicrobial essential oils cause structural and functional damages of microbes by disrupting of membrane permeability and osmotic balance of the cell. Phenolic compounds play the primary role (Palou *et al*., 2008; Prakash *et al.*, 2015).

Conclusion

In recent years, the need to develop plant based food preservatives as alternative to synthetic chemicals to control qualitative and quantitative losses of food item by moulds, mycotoxin contamination has become a priority of scientists worldwide (Prakash *et al.*, 2015). In this context our results revealed a part of the high potential of plant extracts to control fungal diseases in foods. Therefore this will constitute a bio-alternative to the use of chemical pesticides or preservatives as they are natural biodegradable recommended free radical scavengers. Additional studies are required to elucidate plant *in vivo* effects and setting foundations for the public use.

References

Ameziane, N., Boubaker, H., Boudyach, H., Msanda, F., Jilal, A. and Ait Benaoumar, A. 2007. Antifungal activity of Moroccan plants against citrus fruit pathogens. Agronomy for Sustainable Development 27: 273-277.

Aplsarlyakul, A., Vanittanakom, N. and Buddhasukh, D. 1995. Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae). Journal of Ethnopharmacology 49: 163-169.

Ariza, M.R., Larsen, T.O., Petersen, B.O., Duus, J.O. and Barrero, A.F. 2002. P*enicillium digitatum* metabolites on synthetic media and citrus fruits. Journal of Agricultural and Food Chemistry 50: 6361-6365.

Askarne, L., Talibi, I., Boubaker, H., Boudyach, E.H., Msanda, F., Saadi, B. and Ait Ben Aoumar, A. 2013. Use of Moroccan medicinal plant extracts as botanical fungicide against citrus blue mould. Letters in Applied Microbiology 56(1): 37-43.

Atik Bekkara, F., Bousmaha, L., Taleb Bendiab, S.A., Boti, J.B. and Casanova, J. 2007. Composition chimique de l'huile essentielle de *Rosmarinus officinalis* L poussant à l'état spontané et cultivé de la région de Tlemcen. Biologie and Santé 7(1): 6-11.

Benjilali, B., Tantaoui-Elaraki, A., Ismaïli-Alaou, M. and Avadi, A. 1986. Méthode d'étude des propriétés antiseptiques des huiles essentielles par contact direct en milieu gélosé. Plantes médicinales et phytothérapie 20(2) :155-167.

Biche, M. 2012. Les principaux insectes ravageurs des agrumes en Algérie et leurs ennemis naturels. GTFS/ REM/070/ITA.

Bousbia, N., Abert, M., Ferhat, M., Petitcolas, E., Meklati, B. and Chemat, F. 2009. Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity. Food Chemistry 114: 355-362.

Boutekedjiret, C., Bentahar, F., Belabbes, R. and Bessiere, J.M. 1998. The essential oil from *Rosmarinus officinalis* L. in Algeria. Journal of Essential Oil Research 10(6): 680-682.

Boutekedjiret, C., Bentahar, F., Belabbes, R. and Bessiere, J.M. 2003. Extraction of rosemary essential oil by steam distillation and hydrodistillation. Flavour and Fragrance Journal 18: 481-484.

Buron-Moles, G., López-Pérez, M., González-Candelas, L., Viñas, I., Teixidó, N., Usall, J. and Torres, R. 2012. Use of GFP-tagged strains of *Penicillium digitatum* and Penicillium expansum to study host-pathogen interactions in oranges and apples. International Journal of Food Microbiology 160: 162-170.

Centeno, S., Calvo, M.A., Adelantado, C. and Figueroa, S. 2010. Antifungal activity of extracts of *Rosmarinus officinalis* and *Thymus vulgaris* against *Aspergillus flavus* and *A. ochraceus*. Pakistan Journal of Biological Sciences 13(9): 452-455.

Djeddi, S., Bouchenah, N., Settar, I. and Skaltsa, H.D. 2007. Composition and antimicrobial activity of the essential oil of *Rosmarinus officinalis* from Algeria. Chemistry of Natural Compounds 43(4): 487-490.

Erkan, N., Ayranci, G. and Ayranci, E. 2008. Antioxidant activities of rosemary (*Rosmarinus officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chemistry 110: 76-82.

Esmaeili, M.A. and Sonboli, A. 2010. Antioxidant, free radical scavenging activities of *Salvia brachyantha* and its protective effect against oxidative cardiac cell injury. Food and Chemical Toxicology 48: 846-853.

Feng, W., Chen, J., Zheng, X. and Liu, Q. 2011. Thyme oil to control Alternaria alternata *in vitro* and *in vivo* as fumigant and contact treatments. Food Control 22: 78-81.

Ghezli, C. and Aouane, B. 2002. Evaluation des maladies des agrumes transmissibles par greffage sur le matériel végétal de multiplication de l'ITAF. In D'Onghia, A.M., Djelouah, K., Roistacher, C.N. (Eds). Proceedings of the Mediterranean research network on certification of citrus (MNCC): 1998-2001. Bari : CIHEAM p. 101-104 (Options Méditerranéennes: Série B. Etudes et Recherches; n. 43).

Gulluce, M., Sahin, F., Sokmen, M., Ozer, H., Daferera, D., Sokmen, A., Polissiou, M., Adiguzel, A. and Ozkan, H. 2007. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. Longifolia. Food Chemistry 103: 1449-1456.

Haddouchi, F., Lazouni, H.A., Meziane, A. and Benmansour, A. 2009. Etude physicochimique et microbiologique de l'huile essentielle de Thymus fontanesii Boiss & Reut. Afrique science 05(2): 246-259.

Hatipoglu, G., Sökmen, M., Bektas, E., Daferera, D., Sökmen, A., Demir, E. and Sahin, H. 2013. Automated and standard extraction of antioxidant phenolic compounds of *Hyssopus officinalis* L. ssp. Angustifolius. Industrial Crops and Products 43: 427-433.

Hendel, N., Larous, L., Sari, M., Boudjelal, A. and Sarri, Dj. 2012. Place of Labiates in folk medicine of the area of M'sila (Algeria). Global Journal of Research on Medicinal Plants and Indigenous Medicine 1(8): 315-322.

Hosni, K., Hassen, I., Chaâbane, H., Jemli, M., Dallali, S., Sebei, H. and Casabianca, H. 2013. Enzyme-assisted extraction of essential oils from thyme (*Thymus capitatus* L.) and rosemary (*Rosmarinus officinalis* L.): Impact on yield, chemical composition and antimicrobial activity. Industrial Crops and Products 47: 291-299.

Hussain, A.I., Anwar, F., Chatha, S.A.S., Jabbar, A., Mahboob, S. and Nigam, P.S. 2010. Rosmarinus officinalis essential oil: antiproliferative, antioxidant and antibacterial activities. Brazilian Journal of Microbiology 41: 1070-1078.

Jamshidi, R., Afzali, Z. and Afzali, D. 2009. Chemical composition of hydrodistillation essential oil of rosemary in different origins in Iran and comparison with other countries. American-Eurasian Journal of Agricultural and Environmental Sciences 5(1): 78-81.

Kerboua, M. 2002. L'agrumiculture en Algérie. In D'Onghia, A.M., Djelouah, K., Roistacher, C.N. (Eds). Proceedings of the Mediterranean research network on certification of citrus (MNCC): 1998-2001. Bari: CIHEAM p. 21-26 (Options Méditerranéennes: Série B. Etudes et Recherches; n. 43).

Khlifi, D., Sghaier, R., Amouri, S., Laouini, D., Hamdi, M. and Bouajila, J. 2013. Composition and antioxidant, anticancer and antiinflammatory activities of *Artemisia herba* alba, *Ruta chalpensis* L. and *Peganum harmala* L. Food and Chemical Toxicology 55: 202-208.

Kocić-Tamackov, S.D. and Dimić, G.R. 2013. Antifugal activity of essential oils in the control of foodborne fungi growth and mycotoxine biosynthesis in food. In A. Méndez-Vilas (Eds). Microbial pathogens and strategies for combating them: science, technology and education, p. 838-849.

Marandi, R.J., Hassani, A., Ghosta, Y., Abdollahi, A., Pirzad, A. and Sefidkon, F. 2011. Control of *Penicillium expansum* and *Botrytis cinerea* on pear with *Thymus kotschyanus, Ocimum basilicum* and *Rosmarinus officinalis* essential oils. Journal of Medicinal Plants Research 5(4): 626-634.

Mekbib, S.B., Regnier, T.J.C. and Korsten, L. 2011. Efficacy and mode of action of yeast antagonists for control of *Penicillium digitatumin* oranges. Tropical Plant Pathology 36(4): 233-240.

Miladi, H., Ben Slama, R., Mili, D., Zouari, S., Bakhrouf, A. and Ammar, E. 2013. Essential oil of Thymus vulgaris L. and Rosmarinus officinalis L.: Gas chromatography-mass spectrometry analysis, cytotoxicity and antioxidant properties and antibacterial activities against foodborne pathogens. Natural Science 5: 729-739.

Moreno, S., Scheyer, T., Romano, C.S. and Vojnov, A.A. 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radical Research 40: 223-231.

Naqvi, S.A.M.H. 2004. Diseases of fruits and vegetables, Volume I. S.A.M.H. Naqvi (Ed.). Kluwer Academic Publishers.

Neri, F., Mari, M. and Brigati, S. 2006. Control of Penicillium expansum by plant volatile compounds. Plant Pathology 55: 100-105.

Nguefack, J., Lekagne Dongmo, J.B., Dakole, C.D., Leth, V., Vismer, H.F., Torp, J., Guemdjom, E.F.N., Mbeffo, M., Tamgue, O., Fotio, D., Amvam Zollo, P.H. and Nkengfack, A.E. 2009. Food preservative potential of essential oils and fractions from *Cymbopogon citratus, Ocimum gratissimum* and *Thymus vulgaris* against mycotoxigenic fungi. International Journal of Food Microbiology 131: 151-156.

Nuzhat, T. and Vidyasagar, G.M. 2013. Antifungal investigations on plant essential oils. A review. International Journal of Pharmacy and Pharmaceutical Sciences 5(2): 19-28.

Ojeda-Sana, A.M., Van Baren, C.M., Elechosa, A.M., Juárez, M.A. and Moreno, S. 2013. New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components. Food Control 31: 189-195.

Okoh, O.O., Sadimenko, A.P. and Afolayan, A.J. 2011. Antioxidant activities of *Rosmarinus officinalis* L. essential oil obtained by hydrodistillation and solvent free microwave extraction. African Journal of

Biotechnology 10: 4207-4211.

Ouibrahim, A., Tlili-Ait-kaki, Y., Bennadja, S., Amrouni, S., Djahoudi, A. G. and Djebar, M.R. 2013. Evaluation of antibacterial activity of *Laurus nobilis* L., *Rosmarinus officinalis* L. and *Ocimum basilicum* L. from Northeast of Algeria. African journal of microbiology research 7(42): 4968-4973.

Palou, L., Smilanick, J.L. and Droby, S. 2008. Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. Stewart Postharvest Review 4(2): 1-16.

Palou, L., Smilanick, J.L., Usall, J. and Viñas, I. 2001. Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. Plant Disease 85: 371-376.

Pitt, J.I. and Honking, A.D. 2009. Fungi and Food Spoilage. 3rd edn. Springer Science and Business Media.

Platania, C., Restuccia, C., Muccilli, S. and Cirvilleri, G. 2012. Efficacy of killer yeasts in the biological control of *Penicillium digitatum* on Tarocco orange fruits *(Citrus sinensis)*. Food Microbiology 30: 219-225.

Prakash, B., Kedia, A., Mishra, P.K. and Dubey, N.K. 2015. Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agrifood commodities - Potentials and challenges. Food Control 47: 381-391.

Prakash, B., Singh, P., Kedia, A. and Dubey, N.K. 2012. Assessment of some essential oils as food preservatives based on antifungal, antiaflatoxin, antioxidant activities and *in vivo* efficacy in food system. Food Research International 49: 201-208.

Rababah, T.M., Hettiarachchy, N.S. and Horax, R. 2004. Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and tert-Butylhydroquinone. Journal of Agricultural and Food Chemistry 52(16): 5183-5186.

Ruberto, G. and Baratta, M.T. 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chemistry 69: 167-174.

Santos, R.D., Shetty, K., Cecchini, A.L. and Miglioranza. L.H.S. 2012. Phenolic compounds and total antioxidant activity determination in rosemary and oregano extracts and its use in cheese spread. Semina: Ciências Agrárias 33(2): 655-666.

Sourov, K., Mustahsan, B., Masud, R., Zannatul, N., Mahmodul, I., Hasanuzzaman, Md., Ramjan, A. and Rana, B. 2014. Phytochemical and *in vitro* biological investigations of methanolic extracts of Enhydra fluctuans Lour. Asian Pacific Journal of Tropical Biomedicine 4: 299-305.

Suhaj, M. 2006. Spice antioxidants isolation and their antiradical activity: a review. Journal of Food Composition and Analysis 19: 531-537.

Talibi, I., Askarne, L., Boubaker, H., Boudyach, E.H., Msanda, F., Saadi, B. and Ait Ben Aoumar, A. 2012. Antifungal activity of Moroccan medicinal plants against citrus sour rot agent Geotrichum candidum. Letters in Applied Microbiology 55(2):155-161.

Tepe, B., Donmez, E., Unlu, M., Candan, F., Daferera, D., Vardar-Unlu, G., Polissiou, M. and Sokmen, A. 2004. Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chemistry 84: 519-525.

Tripathi, P. and Dubey, N.K. 2004. Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables, a review. Postharvest Biology and Technology 32: 235-245.

Viñas, I., Vallverdú, N., Monllaó, S., Usall, J., Sanchis, V. 1993. Imazalil resistant *Penicillium* isolates from Spanish apple packinghouses. Mycopathologia 123: 27-33.

Wang, W., Wu, N., Zu, Y.G. and Fu, Y.J. 2008. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chemistry 108: 1019-1022.

Wojdyło, A., Oszmianskii, J. and Czemerys, R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry 105: 940-949.

Wong, S.P., Leong, L.P. and Koh, J.H.W. 2006. Antioxidant activities of aqueous extracts of selected plants. Food Chemistry 99: 775-83.

Yesil-Celiktas, O., Bedir, E. and Vardar Sukan, F. 2007. *In vitro* antioxidant activities of *Rosmarinus officinalis* extracts treated with supercritical carbon dioxide. Food Chemistry 101: 1457-1464.

Yesil-Celiktas,O., Hames Kocabas, E.E., Bedir, E., Verdar Sukan, F., Ozek,T. and Baser, K.H.C. 2007a. Antimicrobial activities of methanol extract and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. Food Chemistry 100: 553-559.

Yesil-Celiktas, O., Girgin, G., Orhan, H., Wichers, H.J., Bedir, E.and Vardar-Sukan, F. 2007b. Screening of free radical scavenging capacity and antioxidant activities of *Rosmarinus fficinalis* extracts with focus on location and harvesting times. European Food Research and Technology 224: 443-51.

Yigit, F., Ozcan, M. and Akgul, A. 2000. Inhibitory effect of some spice essential oils on *Penicillium digitatum* causing postharvest rot in citrus. Grasas y Aceites 51(4): 237-240.

Zabka, M., Pavela, R. and Slezakova, L. 2009. Antifungal effect of *Pimenta dioica* essential oil against dangerous pathogenic and toxinogenic fungi. Industrial Crops and Products 30: 250-253.

Zamani, M., Sharifi Tehrani, A., Ahmadzadeh, M., Hosseininaveh, V. and Mostofy, Y. 2009. Control of *Penicillium digitatum* on orange fruit combining Pantoea agglomerans with hot sodium bicarbonate dipping. Journal of Plant Pathology 91(2): 437-442.

Zaouali, Y., Bouzaine, T. and Boussaid, M. 2010. Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities. Food and Chemical Toxicology 48: 3144-3152.