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The effect of *Cotula cinerea* essantial oil on spore germination, mycelial growth and on sporulation of phytopathogenic fungi : *Aspergillus niger*

Benahmed Fatiha [1]*, Djebbar Nadjat [1]

[1]Faculty of Nature and Life Sciences, Department of Biology, Tahri Mohamed University of Bechar, Bechar (08000), Algeria,

*Corresponding author: biologie_biologie@yahoo.fr

Abstract – This work aimed to evaluate the antifungal activity of *Cotula cinerea* essential oil against *Aspergillus niger*. Essential oil from the aerial parts of the plant was obtained by hydrodistillation and the antifungal activity was investigated by agar dilution method on spore germination, mycelial growth and on sporulation of *A. niger* using different concentrations (1/100, 1/250, 1/500, 1/1000, 1/2000, 1/5000 and 1/10000 (v/v)). Results showed that the essential oil yield was 1,42% (v/w). The results from this study demonstrated that oil extract was effective in inhibiting spore germination, radial growth and sporulation of *A. niger*. The inhibitory effect of extract increase when concentration increased. Complete inhibition of conidial germination and mycelial growth was found at 1/250 and 1/100 respectively. But in case of sporulation, 80% was the most inhibition rate. This study suggests that the essential oil of aerial parts of *C. cinerea* have antifungal activity and could be an al-ternative treatment in controling *A. niger*.

Keywords: Antifungal properties, *Aspergillus niger*, *Cotula cinerea*, Essential oil.

1. Introduction

Different food products can be contaminated by various filamentous fungi, leading to important economic losses. Fungal infection leads to food deterioration like off-flavors, discoloration, rotting and disintegration of the food structure. One of these fungi is *Aspergillus.* The genus *Aspergillus* includes over 185 species which are ubiquitous and are especially common in soil and decaying vegetation (Uniyal et al., 2012), but, *Aspergillus niger* is the most common contaminants of food (Frisvad et al., 2011), especially fruits and certain vegetables and is frequently determined in grapes, green coffee beans, onions, mango, maize and other cereals, peanuts, dried fruits, and many other products (Pitt and Hocking, 1997). In order to reduce the effects of the ingestion of food contaminated with fungi, different strategies applied aimed to avoid contamination of fungi. Many chemical preservatives that target fungi growth in food have been approved. Recently the consumers are looking and demanding for products without chemical preservatives and still maintain good shelf life and safe (Muhialdin and Zaiton, 2011). For these reasons, there are current studies on the application of essential oils, extracts, oleoresins and their components extracted from spices and other aromatic plants, as alternative biopreservatives (Hsieh et al., 2001; Kocić-Tanackov et al., 2012). Several authors have indicated that some products containing essential oils from spices and herbs are an alternative for controling the microbial growth in foods

for human consumption (Govaris et al., 2010; Hulankova et al., 2013).

The antimicrobial effect of different essential oils is known for many centuries. Several essential oils and their constituents were investigated for their antimicrobial activities against numerous bacteria and fungi strains (Alizadeh *et al*., 2010; Bansod and Rai, 2008). The selection of plants for evaluation was based on traditional usage for treatment of infection diseases (Panizzi *et al.,* 1993). In this contexte, researches on *Cotula cinerea* (Asteraceae) were initiated. This plant has been extensively used in traditional medicine for treatment for bronchopulmonary diseases, digestive problems, sunstroke and colic (Mebarki, 2016). The studies concerning the antifungal activity of the essential oils of *C. cinerea* are relatively limited.

In the present study, it has been therefore thought desirable to discover the antifungal activity of essential oil of *C. cinerea* against *A. niger*.

2. Materials and Methods

2.1 Plant material and essential oil extraction

Aerial part of *C. cinerea* was harvested from the area of Bechar (Southwest of Algeria) at flowering stage in February 2016. The plant was authenticated at biology department, university of Bechar. A specimen was deposited in the herbarium of this department. Plant material was air dried in the shade for few days.

The air dried aerial part was hydrodistilled in Clevenger's apparatus for 3h (Clevenger, 1928). The yield sample of essential oil was determined after three hydrodistillations of 70g dry material. The obtained essential oil was then kept at dark in 4° C until use.

2.2 Test organism

The strain of *A. niger* used in this study was obtained from the Laboratory of valorization of vegetal resources and food security in semi-arid area, south-west Algeria, University of Bechar. The fungal culture was maintained and grown on Potato Dextrose Agar (PDA) medium at 4 C°. A 7-14 days old culture of the isolate was used as the source of inoculum and for the preparation of spore suspension for different studies.

2.3 Antifungal assays

Antifungal activity of *C. cinerea* essential oil was investigated by agar dilution method on spore germination, mycelial growth and on sporulation of *A. niger*.

2.4 Effect of essential oil on spore germination

The antifungal activity on spore germination of *A. niger* was performed in sterile Petri dishes containing sterile potato dextrose agar (PDA) mixed with essential oil to obtain final concentrations 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/5000 and 1/10000 (v/v). Then, 0.1 ml of a spore suspension (10^5 spores/ml) of *A. niger*, prepared in sterile physiological water was spread on the mixture (PDA + essential oil). The Petri dishes were incubated at 25 °C for 24 h. Counting of spores germinated or ungerminated was determined microscopically on a total of 200 spores. A spore is considered germinated if the germ tube length is greater than its diameter. Control tests were performed under the same conditions in absence of essential oil (Maouni *et al.,* 2001).

2.5 Effect of essential oil on mycelial growth (Minimal inhibitory concentration: MIC)

The essential oil was added aseptically to a sterile molten PDA medium to obtain final concentrations 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/5000 and 1/10000 (v/v). The resulting PDA solutions were immediately poured into Petri plates after vortexing. Six millimeter mycelial discs were taken from the margins of 7 days old culture of the tested fungi and placed on the middle of a PDA plate. The colony diameter was then measured after incubation at 25 °C for 07 days. Two measurements from each plate were taken at a 90° angle from each other. The two measurements were averaged to determine the diameter of the fungal colony.

The MIC was determined as the lowest concentration of oil inhibiting the visible growth of *A. niger* on the agar plate. Control tests were performed under the same conditions in absence of essential oil (Remmal *et al.*, 1993; Satrani *et al.*, 2001).

2.6 Effect of essential oil on sporulation

All colonies used to assess mycelial growth were reincubated until the 10^{th} day at 25 °C for evaluating the effect of the essential oil on sporulation. For this, 4 washers measuring 5 mm in diameter were taken along the diameter of the same colony and were collected in a tube containing 1 ml of sterile distilled water. After crush washers and agitation to the vortex for 30 s, the spores were counted using a Malassez cell with three counts by suspension (Maouni *et al.*, 2001).

2.7 Assessment of the antifungal activity

The percentage of inhibition was calculated using the following formula (Amadioha, 2003):

Inhibition (%) = (C - T). 1OO/C.

Where C and T represent spores germination (radial growth or sporulation) in control and treated plates, respectively.

3. Results

3.1 Kinetic of essential oil extraction

Extraction kinetics consists in determining the volume of the essential oil depending on the extraction time. This experience aims to set the time required to extract the maximum of oil and to avoid loss of time. The kinetic of essential oil extraction of 70g of dry aerial part of *C. cinerea* indicates that the volume of the oil increases rapidly with time then it stabilizes from 50min at 1ml of volume (figure 1). The essential oil yield was 1,42% (v/w) and it was 0,97% (w/w).

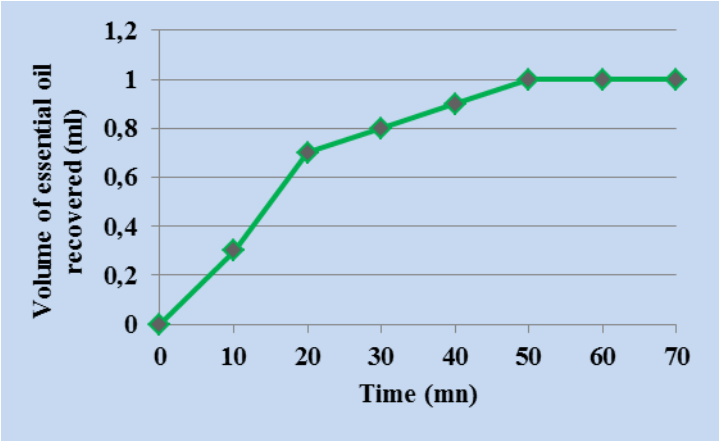

Figure 1. Kinetics of essential oil extraction (for 70g).

3.2 Antifungal activity of essential oil

In this study, *C. cinerea* essential oil was evaluated for his inhibitory activity on spore germination, mycelial growth and on sporulation of *A. niger*.

C. cinerea essential oil was tested as inhibitor against conidial germination of *A. niger*. After 24 hour of incubation, spore germination was inhibited at all essential oil concentrations tested (figure 2). The effects ranged from minor inhibition to

complete inhibition of germination. The percentage of inhibition was low at small concentrations. The lowest inhibitory effect was 03,57% and it was observed at the lowest concentration. The MIC value that caused 100% inhibition of conidial germination was 1/250.

At all concentrations tested, the colony diameter of *A. niger* was reduced by essential oil of *C. cinerea* compared to the control. It was observed that the inhibitory effect increased as essential oil concentration increased. Results showed that the lowest inhibition (14,75%) was found at 1/10000 and essential oil concentrations of 1/250 was inhibited the mycelia growth of *A. niger* by 50,80% (Figure 3). However**,** Complete inhibition of mycelia growth was observed at the concentration of 1/100. Indeed, the transfer of mycelial discs where growth inhibition was complete by *A. niger* into PDA medium without essential oil, showed mycelial growth after some days of incubation, indicating a fungistatic effect for this oil at 1/100 concentration.

Effect of essential oil on sporulation of tested fungi was examined. The results in figure 4 showed that the sporulation was inhibited at all concentration and the inhibitory effect increased as concentration increased. It was observed that the sporulation inhibition ranged from 06 to 56,83% by application of essential oil at the concentration range of 1/10000 to 1/250. In fact, the highest inhibition (80%) was found at the highest concentration on comparison with PDA (control).

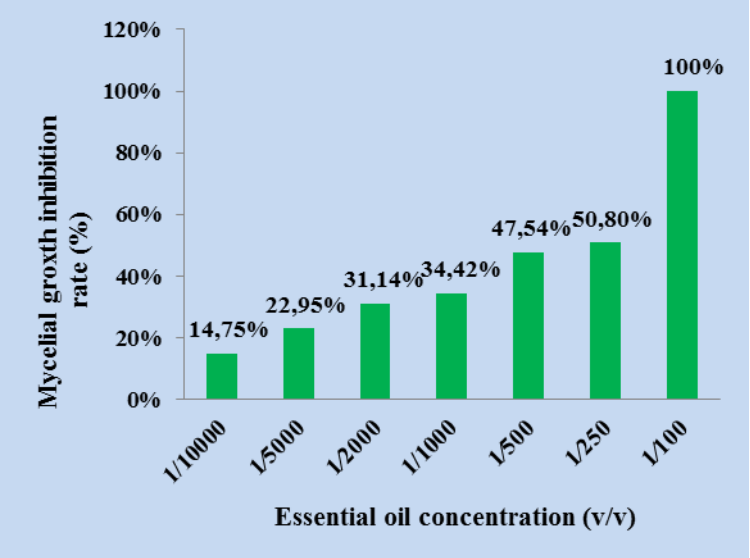

Figure 2. Effect of essential oil on spore germination.

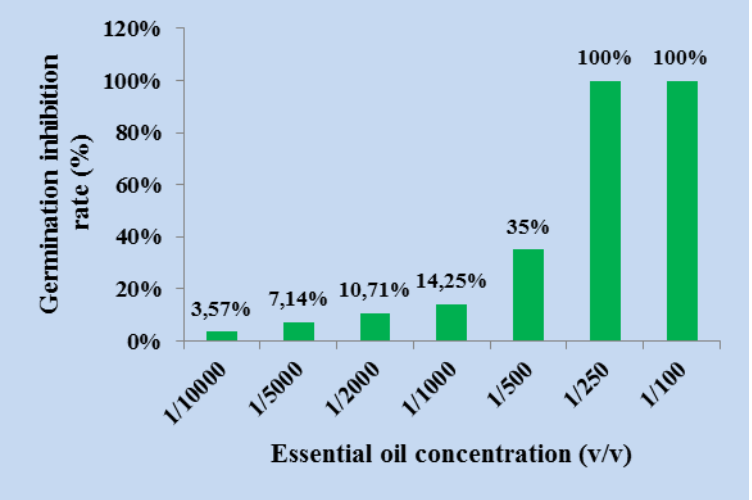

Figure 3. Effect of essential oil on mycelial growth

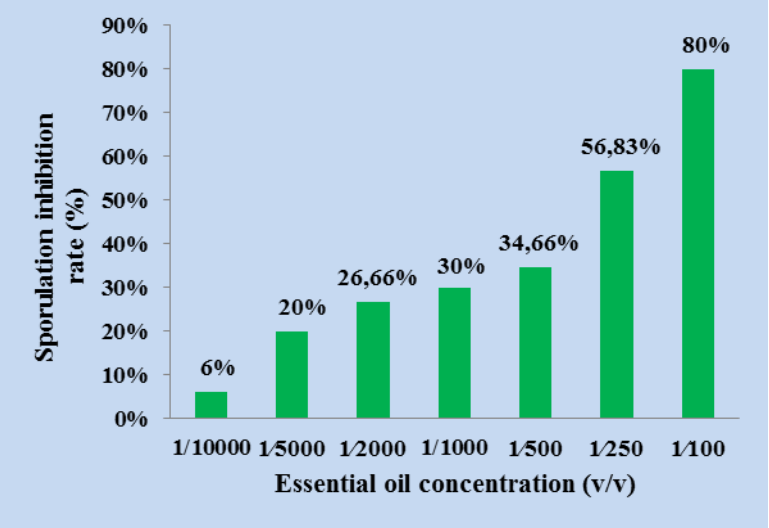

Figure 4. Effect of essential oil on sporulation .

4. Discussion

Medicinal plant evaluated in this study was selected for his previously reported antimicrobial properties in pharmaceutial and food applications. In this study the essential oil of *C. cinerea* was extracted and tested against *A. niger*. Antifungal effects were treated by agar dilution method on spore germination, mycelial growth and on spore production.

The essential oil yield recovered in this study is relatively higher than those obtained by Djellouli *et al*, (2015) and Chouikh *et al*, (2015), which have obtained 0.282% and 0.0801% (v/w) from the aerial part of *C. cinerea* harvested in the area of Bechar (Southwest of Algeria) and in Oued Souf Sahara (South East of Algeria) respectively, but is low than this obtained by Abdenbi *et al*, (2014) (2%) working on the same part of the same plant recolted in the Southwest of Algeria.

In this investigation essential oil exhibited considerable antifungal properties against *A. niger*. The results indicated that the effect was dose dependently. Complete inhibition of conidial germination and mycelial growth was found at 1/250 and 1/100 respectively. But in case of sporulation, 80% was the most inhibition rate.

The antifungal activities of this essential oil observed against *A. niger*, could be attributed to its chemical profile, characterized by its principal components, especially (E)-citral (24.01%), cis-limonene epoxide (18.26%), thymol methyl ether (15.04%), carvacrol (15.03%), trans-carveol (13.79%), carvone (3.06%) and trans-piperitol (2.54%) which were identified by Djellouli *et al*, (2015) in their phytochemical screening of the aerial part essential oil of *C. cinerea* harvested from Bechar area. Also, the antifungal effects could be attributed to the synergy phenomenon between these volatile constituents whose interactions may be the origin of an activity much more pronounced than that expected for the majority of compounds (Rhafouri *et al.*, 2014*;* Bhanu *et al.*, 2016).

It should be noted that various publications have documented the antifungal activity of many other essential oils against *A. niger*, as it is proved by Rhafouri *et al.* **(**2014), Bansod and Rai (2008) and others, but, no study had exhibited the effect of *C. cinerea* essential oil against this tested fungi.

To clarify the mechanism of action of essential oils many studies were performed. Park *et al*, (2009) in their research concerning the effect of citral (major compound of *C. cinerea* essential oil) against *Trichophyton mentagrophytes* found that citral was able to significantly inhibit mycelial growth. Antifungal activity was attributed to cell membrane disruption and to consequent loss of cellular components. Another study also showed that citral at a concentration of 200 µg/mL irreversibly damaged cell organelles and the cell membrane of *Trichophyton mentagrophytes.* In addition, Viuda-Martos *et al*, (2008) suggest that the essential oil components act on the functionality and the structure of the cell membrane. Also, low concentrations result in changes of the cell structure, inhibiting respiration and changing the permeability of the cell membrane, whereas high concentrations lead to severe membrane damage, loss of homeostasis and cell death (*Carson et al.*, 2002). The possible mechanism of action of essential oil components on the growth of fungi was reported in others studies. It is generally agreed that the suppression of fungal growth is associated with the degeneration

of fungal hypha following treatment with different essential oils, these degeneration modifications mainly included cytoplasmic coagulation, vacuolations, the markedly shriveled and crinkled hypha, plasma membrane disruption and hypha wall thickness (Soylu *et al.*, 2006*;* Liu *et al*, 2009; Tian *et al*, 2011). Moreover, Conner and Beuchat (1984) suggest that the antifungal activity is the product of essential oil components' interaction with enzymes responsible for energy production and the synthesis of structural compounds of the cell. On the other hand, (Omidbeygi *et al.*, 2007) suggest that the essential oil components pass through the cell membrane, integrating with enzymes and proteins of membranes, causing loss of macromolecules from the interior of the cell, leading to changes in the cell and ultimately to its death.

5. Conclusion

The most promising information gleaned from this study is the efficacy of the essential oil of *C. cinerea* in inhibiting development of *A. niger* suggesting its potential application as a strong antimicrobial agent within the food, stored products, and cosmetic industry.

6. References

Abdenbi A, Abdelwaheb D.J, Bouaaza M, Touati B. (2014): Screening Phytochimique Et Activité Antibactérienne De L'huile Essentielle De *Cotula Cinerea* (Gartoufa) Dans La Région De Bechar. Int J Res Eng Tec. 2: 50-54.

Alizadeh A, Zamani E, Sharaifi R, Javan-Nikkah M, Nazari S. (2010): Antifungal activity of some essential oils against toxigenic Aspergillus species. Communun Agr Appl Biol Sci. 75(4): 761-767.

Amadioha A.C. Evaluation of some plant extracts against *Colletotrichum lindemnthianum* in cowpea. Acta. Phytopathol. Entomol. Hung (2003); 38: 259-265.

Bansod S, Rai M. (2008): Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *A. niger*. World J Med Sci. 3(2): 81-88

Bhanu P, akash K, aakanksha S, shashi Y, arti S, amrita Y, deepika, nawal kishore D. (2016): Antifungal, antiaflatoxin and antioxidant activity of plant essential oils and their in vivo efficacy in protection of chickpea seeds. J Food Quality. 39 (01) 36–44.

Carson C.F, Mee B.J, Riley T.V. (2002): Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. Antimicrob Agents Ch. 46:1914-1920.

Chouikh A, Boualem M, Cherif M.M, Youcef H, Azzedine C. (2015): Chemical composition and antimicrobial activity of essential oils in xerophytic plant *Cotula cinerea* Del (Asteraceae) during two stages of development: flowering and fruiting. J Appl Pharm Sci. 5(3): 29-34.

Clevenger J.F. (1928): Apparatus for the determination of volatile oil. J. Am. Pharm. Assoc. 17: 345-349.

Conner D.E, Beuchat L.R. (1984): Effect of essential oils from plants on growth of food spoilage yeasts. J Food Sci. 49:429-434.

Djellouli M, Benmehdi H, Mammeri S, Moussaoui A, Zaine L, Hamidi N. (2015): Chemical constituents in the essential oil of the endemic plant *Cotula cinerea* (Del.) from the southwest of Algeria. Asian Pac J Trop Biomed. 5(10): 870–873

Frisvad J.C, Larsen T.O, Thrane U, Meijer M, Varga J, Samson RA, Nielsen K.F. (2011): Fumonisin and ochratoxin production in industrial *Aspergillus niger* strains. Plos One. 6(8):e23496.

Govaris A, Solomakos N, Pexara A, Chatzopoulou P.S. (2010): The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella enteritidis* in minced sheep meat during refrigerated storage. Int. J. Food Microbiol. 137: 175-180.

Hsieh P.C, Mau J.L, Huang S.H. (2001): Antimicrobial effect of various combinations of plant extracts. Food Microbiol. 18:35- 43.

Hulankova R, Borilova G, Steinhauserova I. (2013): Combined antimicrobial effect of oregano essential oil and caprylic acid in minced beef. Meat Sci. 95: 190-194.

Kocić-Tanackov S, Dimić G, Pejin D, Mojović Lj, Pejin J, Tanackov I. (2012): The inhibitory effect of oregano extract on the growth of *Aspergillus* spp. and on sterigmatocystin biosynthesis. LWT – Food Sci Technol. 49:14-20.

Liu X, Wang L.P, Li Y.C, Li H.Y, Yu T, Zheng X.D. (2009): Antifungal activity of thyme oil against *Geotrichum citri-aurantii* in vitro and in vivo. J Appl Microbiol. 107: 1450–1456.

Maouni A, Lamarti A, Douira A, Badoc A. (2001): Effet de dérivés calciques sur le développement de moisissures lors de la conservation des poires. Bull. Soc. Pharm. Bordeaux. 140:79-88.

Mebarki L. (2016): Recherche d'activité biologique de molécules végétales pour la lutte contre *Fusarium oxysporum* f. sp. *albedinis*. Phd thesis, University of Sciences and Technology, Mohamed Boudiaf (USTOMB), Algeria. 54 P.

Muhialdin B.J, Zaiton H. (2011): Screening of Lactic Acid Bacteria for Antifungal Activity against *Aspergillus oryzae*. Am J Appl Sci. (5): 447-451.

Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi H. (2007): Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. Food Control. 18:1518–23.

Panizzi L, Flamini G, Cioni P.L, Morelli I. (1993): Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae., J. Ethnopharmacol. 39: 167-170.

Park M.J, Gwak K.S, Yang I, Kim K.W, Jeung E.B, Chang J.W, Choi I.G. (2009): Effect of citral, eugenol, nerolidol and α-terpineol on the ultrastructural changes of Trichophyton mentagrophytes. Fitoterapia. 80(5): 290–296.

Pitt J.I, Hocking A.D. (1997): Fungi and food spoilage; 2nd ed.; Blackie Academic and Professional: London. 592 p

Remmal A, Bouchikhi T, Rhayour K, Ettayebi M, Tantaoui-Elaraki A. (1993): Improved method for determination of antimicrobial activity of essential oils in agar medium. J. Essent. Oil Res. 5: 179-184.

Rhafouri R, Strani B, Zair T, Ghanmi M, Aafi A, El Omari M, Bentayeb A. (2014): Chemical composition, antibacterial and antifungal activities of the *Cedrus atlantica* (Endl.) Manettiex Carrière seeds essential oil. Med J Chem. 3(5) : 1034-1043

Satrani B, Farah A, Fechtal M, Talbi M, Blaghen M, Chaouch A. (2001): Composition chimique et activité antimicrobienne des huiles essentielles de *Satureja calamintha* et Satureja alpina du Maroc. Ann. Falsif. Expert. Chim. Toxicol. 94(956) : 241-250.

Soylu E.M, Soylu S, Kurt S. (2006): Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent Phytophthora infestans. Mycopathologia. 161: 119–128.

Tian J, Huang B, Luo X, Zeng H, Ban X, He J, Wang Y. (2011): The control of *Aspergillus flavus* with *Cinnamomum jensenianum* Hand.-Mazz essential oil and its potential use as a food preservative. Food Chem. 130: 520–527.

Uniyal V, Bhatt R.P, Seema S, Amitabh T. (2012): Antifungal activity of essential oils and their volatile constituents against respiratory tract pathogens causing Aspergilloma and Aspergillosis by gaseous contact. J. Appl. & Nat. Sci. 4 (1): 65-70.

Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez J. (2008):Antifungal activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi L.*) and orange (*Citrus sinensis* L.) essential oils. Food Control. 19:1130-8.