

# Inhibitory Effect of Some Spice Essential Oils on *Penicillium digitatum* Causing Postharvest Green Rot in Citrus

S. Louhibi<sup>1,2</sup>, L. Elghadraoui<sup>1</sup>, S. Amiri<sup>2</sup>

<sup>1</sup>Laboratory of Functional Ecology and Environment,, department of life science and earth ,Faculty of Sciences and Technical, B.P. 2202 –, Fès, Immouzer, Morocco

<sup>2</sup>Laboratory of phytopathology, Department of Protection the Plants & Environment, Ecole National of Agriculture, BP S 40 Meknès, Morocco

**Abstract:** The aim of this study was to evaluate the antifungal activity of two species collected in different locations of Morocco against *Penicillium digitatum*, the causal agent of citrus green mold. The *in vitro* antifungal activity of essential oil was determined using the method of agar diffusion assay. The results show that essential oil of *Lavandula hybrida* has an inhibitory capacity of mycelial growth and spore production compared to others essential oils. *Penicillium digitatum*; *Lavandula hybrida*; *in vitro*; antifungal activity; *Artemisia herba alba*

**Keywords:** *Penicillium digitatum*; *Lavandula hybrida*; *in vitro*; antifungal activity; *Artemisia herba alba*

## 1. Introduction

In Morocco citrus play a socio-economic role that is very important. On the economic map, citrus exports is an important source of foreign exchange in the order of 2.5 to 3 billion dirham's a year.

In citrus as in any other agricultural production sector commercial character to the final volume of the production is the most important parameter that determines the interest of the culture of a variety thus the improvement and mastery of production techniques and protection prove necessary for a better profitability

The most important citrus disease that cause commercially significant losses in Morocco (Elkhmass et al.,1994) and worldwide (Eckert and Ears;1989;Holmes and Eckert,1999;Zhu et al 2006) are green mold, caused by *penicillium digitatum*. The chemical control remains by far the control method preferred against all rot mainly due to *Penicillium Spp*. Intensive and repeated use of fungicide protection in post citrus fruits favored the recolte development resistant strains worldwide (Harding,1972; Smoot et brown, 1974) even to the consumer tends to look more natural products, has prompted the research, development and application of new natural products with antimicrobial activities in order to use them as alternative to synthesis products in the field of food industrial.

## 2. Materials and Methods

### Plant Material

Species used in experiment are listed in table 1. They were collected from different locations between Meknes and Azrou These plants have been chosen for their medicinal properties and because it is a very important floristic local heritage and it is largely described as a botanical point of

view, the aerial part of the plant was dried in the shade at room temperature and subjected to a hydro distillation 2h.

Table 1: Species used for distilling essential oils

Common name	Botanical name	Family	Used part
Lavender	<i>Lavandula hybrida</i>	Lamiaceae	Leaves+flowers
Artemisia	<i>Artemisia herba alba</i>	Astéracées	Leaves+flowers

### Return Calculation

The yield of essential oil is defined as the ratio between the mass of E.O obtained after extraction (M') and the mass of the plant material used (M) ( Bssaibis F., Gmira Meziane N and M. ( 2009)). The yield is expressed in percentage and is given by the following formula:

$$\text{Yield (\%)} = M'/M \times 100$$

Yield: E.O yield of dry material;

M' : mass in grams from the dry plant material ;

M : mass of dry plant material used in gram

### Pathogen culture

In this study the fungus employed for assays of antifungal activity was isolated from decayed oranges. The oranges were obtained from conditioning station. The identification was performed based on the study of macroscopic and microscopic characters of isolates growing on PDA medium After identifying *Penicillium digitatum*, it was cultured routinely on Potato Dextrose Agar and incubated at 25°C.

### Evaluation of antifungal activity of essential oils:

This effect was tested using aromatoqram method that described by Maychiew and Devahastin (2008) and Hussain and colaboratory (2010), 20 ml PDA cooled are cast in Petri dishes. After solidification of the culture medium, 200 µ.l of the fungal suspension under test (107 spores.ml) were plated on the surface until total desiccation.

Under aseptic conditions and using sterile forceps, Paper filter discs (Whatman Nr 3, 6 mm diameter) ( 1 disque / Petri

dish) are sterilized and then deposit on the dried agar inoculated beforehand with the fungal suspensions then these discs are loaded by volumes of essential oil ( 10,20,30,50,60,80 $\mu$ l ) using a micropipette for each volumes, 3 repetitions were performed to minimize the risk of error.

The Petri dishes are kept at 4°C for 2 hours so that the essential oil may diffuse into the culture medium. For control Petri plate had no essential oil.

The Petri plates were incubated at 25°C for 5 to 7 days until the growth in the control petri dishes reaches the edges.

**Determination of minimum inhibitory concentration (MIC)**

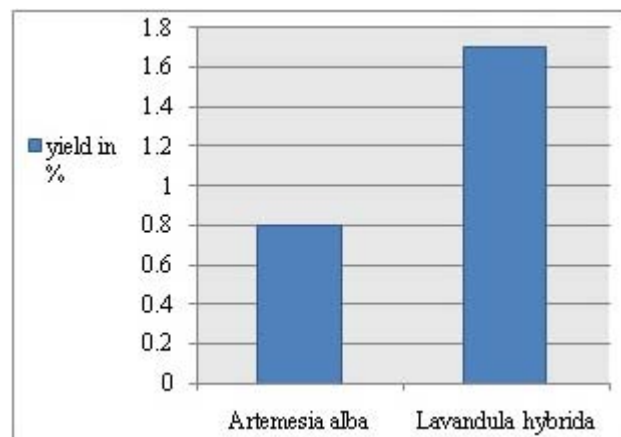
The minimum inhibitory concentrations (MIC) of essential oils were determined according to the method reported by Remmal et al. (1993) and Satrani et al. (2001). Due to the immiscibility of essential oils to the water and thus in the middle of culture, emulsification was carried out through a solution d'agar 0.2%. It provides, in the middle, an even distribution of essential oils to maximize the Contact germ / compound. Dilutions are prepared 1/10th, 1/25th, 1/50th, 1/100th, 1/200th, 1/300th and 1/500th in this agar solution. In test tubes each containing 13.5 ml of medium on PDA (Potato Dextrose Agar) for fungi, autoclaved for 20 min at 121°C and cooled to 45°C, aseptically added 1.5 ml of each dilution to give final concentrations of 1/100, 1/250, 1/500, 1/1 000 1/2 000 1/3 000 1/5 000 (v/v). Stir the tubes to disperse the essential oil in the culture medium before pouring in the Petri dishes.

Petri dishes control, containing the culture medium and the agar solution at 0.2% alone are also prepared

**3. Results and Discussion**

**1-Essential oil yield**

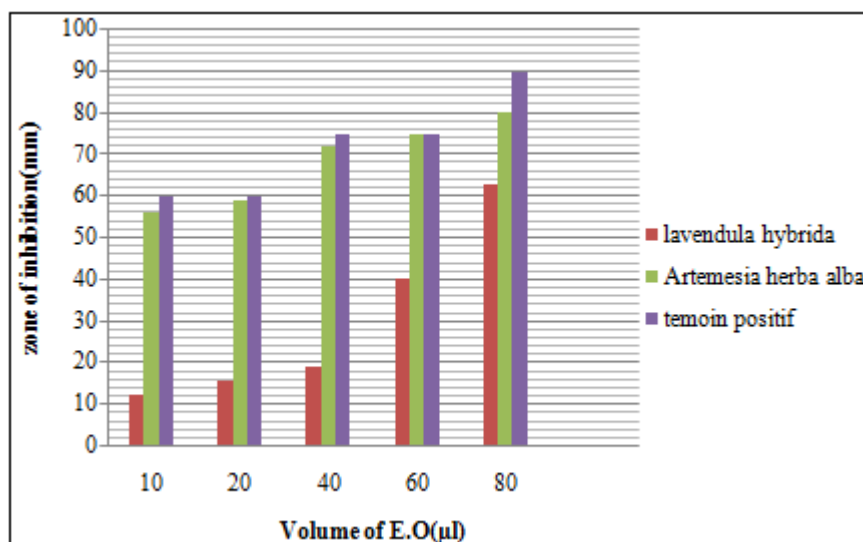
The extraction of E.O by hydro distillation has been used for three plants. The figure shows the yields obtained by E.O extraction plants.



**Figure 1:** Yield of E.O of *Lavandula hybrida* et *Artemisia herba Alba*

This figure shows that the yield of *Lavandula hybrida* is 1.7% which is higher than that of *Artemisia* (0.8 %) The yield of HE, which depends on many factors (growth stage, soil and climatic conditions, extraction technique, harvest etc. ) is only 0.8% for *Artemisia alba*. It can be much higher in the same species, which is about 1.23% ( M.Ghanmi et al, 2010). In this sense Ghanmi confirms that the essential oil content of white mugwort change depending on the date of harvest.

**2- Testing the antifungal activity :**



**Figure 2:** development of the zone of inhibition caused by the essential oil of *Lavandula hybrida* and *Artemisia herba alba*

*P. digitatum* was sensitive to the majority of the tested concentrations of both EO The figure below expresses the change in the average diameters of the inhibition zones (mm) depending on the concentration for the two essential oil, changes in the development of *P. digitatum* is inversely proportional to the increase of the concentration. Also

comparing to the positive control (treatment with imazalil 50%) *Artemisia herba alba* has proved significantly more effective than *lavandula hybrida* especially for the 60 $\mu$ l volume which notes that the inhibition zone and the same diameter as that of the positive control the same for the 80 $\mu$ l dose with a difference of 10  $\mu$ l

### 3-Determination de la CMI:

**Table 1:** The CMI of E.O of *Lavendula* and *Artemesia herba alba*

	Dilution V/V						Control
	1/100	1/250	1/500	1/1000	1/2000	1/3000	
<i>Lavendula hybrida</i>							
<i>Penicillium digitatum</i>	-	-	-	+	+	+	+
<i>Artemesia alba</i>							
<i>Penicillium digitatum</i>	-	-	+	+	+	+	+

(-) Inhibition  
(+) growth

The inhibitory power of essential oils overlooked a microbial strain is classified as: excellent inhibitory power for MIC < 50 µL/ml to interesting inhibitor for CMI 50 µl/ml and 250 µl / ml, low inhibitory power for MIC 250 µl/ml and 500 µl/ ml and to poor or no inhibitor for MICs > 500 µl/ ml ( Koba et al. , 2004). It can be inferred that both essential oils have an interesting inhibitory power on the fungal strains.

### 4. Conclusion

According to these results we can predict that the essential oils of *Lavendula hybrida* and *Artemesia herba alba* are very effective natural antifungals and can be a very important source of herbal constituents used to eradicate infections of fungal origin.

### References

[1] Eckert, J.W., Ears, I.L., 1989. Postharvest Disorders and Diseases of Citrus Fruits. In: The Citrus Industry: Crop Protection, Postharvest Technology, and Early History of Citrus Research in California, vol. 5, 179 p.

[2] Holmes, G.J., Eckert, J.W., 1999. Sensitivity of *Penicillium digitatum* and *P. italicum* to postharvest citrus fungicides in California. *Phytopathology* 89, 716e721.

[3] Zhu, J.W., Xie, Q.Y., Li, H., 2006. Occurrence of imazalil-resistant biotype of *Penicillium digitatum* in China and the resistant molecular mechanism. *J. Zhejiang Univ. Sci.* 7, 362e365.

[4] Pharma copie européenne(2008) huiles essentielles. *Aetherlea* 1 :2098.

[5] Clevenger JF(1928)Apparatus for the determination of volatile oil. *J Am Pharm Assoc* 17: 341-6

[6] Sensitivity of *Penicillium digitatum* and *P. italicum* to Postharvest Citrus Fungicides in California Gerald J. Holmes and Joseph W. Eckert First author: Department of Plant Pathology, North Carolina State University, Raleigh 27695; and second author: Department of Plant Pathology, University of California, Riverside 92521

[7] S. Fellah, M. Romdhane, M. Abderraba, (2006). « Extraction et étude des huiles essentielles de la *Salvia officinalis*. lcueillie dans deux régions différentes de la Tunisie » *J.soc.alger.chim.*, 2006, 16(2), 193-202. journal de la société algérienne de chimie. 16- Felice S. , N

[8] Dorman, H.J.D., Deans, S.G. 2000. "Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.*, 88, 308-316.

[9] François-Xavier Garneau, corporation :LASEVE-UQAC, Chicoutimi (Québec) G7H 2B1 *Huiles essentielles : de la plante à la commercialisation – Manuel pratique* « le matériel végétal et les huiles essentielles ».

[10] Ghanmi M., B. Satrani, A. Aafi, M.R. Isamili, H. Houti, H. El Monfalouti, K.H. Bencheqroun, M. Aberchane, L. Harki,A. Boukir, A. Chaouch, Z. Charrouf. *Phytothérapie* (2010) 8, 295 – 301 : « Effet de la date de récolte sur le rendement, la composition chimique et la bioactivité des huiles essentielles de l'armoise blanche (*Artemisia herba-alba*) de la région de Guercif (Maroc oriental).

[11] Haouari Mohsen - Ferchichi Ali. *Molecules* 2009, 14, 1585-1594: « Essential Oil Composition of *Artemisia herba-alba* from Southern Tunisia".

*Journal of Essential Oil Research*

[12] Mayachiew P. et DEvahastin S.,2008 Antimicrobial and antioxidant activities of indian gooseberry and galangal extracts. *food science and technology* 41 :1153-1159.

[13] Elkhmass, M., Oulahcen, B., Lekchiri, A., Sebbata, A., Charhabaili, Y., 1994. Stratégie de lutte contre les maladies de post-récolte des fruits d'agrumes.

[14] AitOubahou, A., El-Otmani, M. (Eds.), *Postharvest Pathology and Technology for Horticultural Commodities: Recent advances.* Institut Agronomique et Vétérinaire Hassan II, Agadir, Maroc, pp. 388e398

[15] Smoot J.J. et Brown G.E., 1974. Occurrence of benzimidazoles – resistant strains of *Penicillium digitatum* in Florida citrus packinghouses, *Plant Dis. Repr.*, 546 - 554.

[16] Bssaibis F., Gmira N et Meziane M. (2009)- Activité antibactérienne de *Ditrichia viscosa* (L.) W.Greuter. *Rev. Microbiol. Ind. San et Environn.* Vol 3, N°1, p : 44-55

[17] Koba K., Sanda K., Raynaud C., Nenonene Y. A., Millet J et Chaumont J. P. (2004). Activités antimicrobiennes d'huiles essentielles de trois *Cymbopogon* sp. africains vis-à-vis de germes pathogènes d'animaux de compagnie. *Annales de Médecine Vétérinaire.* 148, 202-206.