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Disinfection of Citrus Storage Room by Fumigation with Two Plants and Chemical Product Deccofenato Pot against the Viability of *Penicillium* Spp Spores

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ABSTRACT
Citrus fruits are subject to many postharvest phytopathogenic fungi that cause severe economic losses. The most attacks are mainly due the genus *Penicillium* spp. The treatment of these fungi uses a lot of chemicals that negatively affect environment and human health. Our study aims to test the antifungal activity by fumigation of two plants and a new chemical pesticide at the station Agrumar Souss, Ait Melloul. Plants and pesticide Deccofenato pot were used as fumigation in order to disinfect the storage rooms. The pesticide was fumigated in the storage rooms while the areal parts of *Artemisia herba alba* and the seeds of *Peganum harmala* were fumigated in hermetically closed chamber installed in the storage room. They were burned using the electric resistance, while the pesticide is already formulated as a fumigant. The results obtained show that both the two plants are very effective to reduce the infestation rates caused by *Penicillium* spp. *Artemisia herba alba* and *Peganum harmala* used respectively at the doses of 25 and 35 g/m^3 inhibited 100% the viability of spores of *Penicillium* spp as well as the pesticide used at 1 g/m^3.
***Keywords*:** *Artemisia herba alba*, *Peganum harmala*, storage room, Deccofenato pot, Fumigation, *Penicillium* spp.

I. INTRODUCTION

Penicillium digitatum and *Penicillium italicum* cause losses of about 90% of all losses caused by fungi of postharvest citrus [1]. The control strategy used against these diseases is mainly based on the use of chemical specialties. In order to prevent and inhibit the development of these fungi, only chemical fungicides are currently employed. However, these products are harmful to the environment and human health by the persistence of residues on fruits [2], the emergence of resistant forms [3; 4; 5] and the accumulation fungicides in human adipose tissue [6]. Recognizing this reality, the consumer requires more chemical free foods. It is in this context that it became necessary to test new chemical and biological specialties alternatives to existing ones.

The effectiveness of the smoke of the aerial parts of *Artemisia herba alba* and seeds of *Peganum harmala* has been studied in order to reduce infection by spores of *Penicillium* spp in a cold room of the packing citrus station Agrumar Souss. This efficacy was compared with that of a new chemical used in disinfection of storage room.

II. Material and methods

1.1 Biological material

Artemisia herba alba and *Peganum harmala* were selected for this study because of their ethnobotanical use [7]. The plants were harvested at physiological maturity stage in two different regions: *Artemisia herba alba* in Tizi n'test and *Peganum harmala* in chichaoua. Seeds of *P. harmala* were obtained from the dried globular fruits, whereas *A. herba alba*, aerial parts were dried in a dry and shady place.

1.2 Preparation of culture medium

PDA medium (potato dextrose agar) was sterilized for 20 min at 120 ° C. After autoclaving and cooling of the medium at a temperature of about 45 °C, 15 ml of the solution were pooled into Petri dishes of 90 mm of diameter. Trapping spores was done before and after treatment by fumigation of two plants in hermetically closed chamber and Deccofenato pot in storage rooms. For this we used a trapping apparatus called aerobiocollector on which is deposited the Petri dish containing PDA medium. The aerobiocollector was to 10L of air / min.

1.3 Evaluating the effectiveness of smoke from the aerial parts of A. herba alba and P. harmala against the spores of Penicillium spp

To evaluate the antifungal activity of fumigation of the storage room by burning the aerial parts of *Artemisia herba alba* and the seeds of the *Peganum harmala*, the method of incineration by an electric stove was used. The experimental material consists of two hermetically closed chambers installed in the storage room. They consist of pieces of wood and plastic with a thickness of 220 microns to a volume of 0.5 m^3 raised from soil by 1m. The two chambers were contaminated by depositing 15 by *Penicillium* spp rotten fruit for a period of 5 days. This period was determined after several preliminary tests. The aerial parts of *A. herba alba* or the seeds of *P. harmala* were deposited under galvanized tray on the electric stove 100 KWH. The smoke is directed to the chamber via a PVC tube. A control without plant was also used. After the fumigation of the two chambers by *A. herba alba* aerial parts and *P. harmala* seeds, the chambers have been sealed for a period of 12 hours. The infestation rates were evaluated before and after fumigation. Preliminary tests have allowed us to select doses for studying plants, and the doses used for both plants were: 5; 10; 15; 20, 25, 30 and 35 g/m^3 of air. Four repetitions were prepared for each dose. The Petri dishes were incubated at 25 °C for 5 days.

1.4 Treatment of the storage rooms room with the product Deccofenato pot

Deccofenato pot is a white powder packed in tins of 250 g each. This powder contains the two active ingredients: orthophenylphenol 30 % w / v (300 g / L), 2- phenylphenol and biphenyl-2 -ol, potassium chloride 16 % w / v (160 g / L).

The storage cold rooms are the property of the cooperative Agrumar Souss located in Ait Melloul industrial area. Four rooms No: 7, 8, 9 and 12 were used for the disinfection by Deccofenato pot, each with 1,600 m^3 of volume. The disinfections take place when the rooms are empty. The air trapping for each room was done in four replications using the aerobiocollector. This latter contains Petri dishes with the PDA medium prepared as above. The *Penicillium* spp spores were trapped before and after application of the fungicide. Samples were placed in an incubator at 25 ° C ± 1 ° C for 5 days. The fungicide was applied as recommended by the formulator of this specialty and at the dose of 1 g/m^3 of the air.

1.5 Calculation of the percentage of the infestation

The results reading was done by direct counting the number of colonies developed using a colony counter (UFC/ Petri dishes). The percentage reduction of the infestation by the smoke from each plant at different concentrations and also for the fungicide was determined by the following formula:

$$E(\%) = \frac{X - Xi}{X} \times 100$$

with:
X : Estimation of infestation by *Penicillium* spp in the control .
Xi : Estimation of infestation by *Penicillium* spp in treated (test) .

1.6 Statistical analysis

The collected results are presented as averages of 4 replicates after having statistically analyzed by SPSS 15 software. To determine any differences between treatments, analysis of variance was performed to a single criterion of classification. If there are significant differences, the Newman -Keuls test (P ≤ 0,05%) was used to highlight the homogeneous classes.

III. RESULTS AND DISCUSSION

The results presented in Figure 1 and table 1 show that the fumigation using the aerial parts of *Artemisia herba alba* and the seeds of *Peganum harmala* is very effective to reduce the viability of spores of *Penicillium* spp. *A. herba alba* was more inhibitory of spore viability of *Penicillium* spp than the smoke of the *P. harmala* at the of dose 15 g / m^3. At this dose, the percentage of the infestation reduction was at 98,62 % and 89,67% for *A. herba alba* and *P. harmala* respectively. According to statistical analysis, 100% of the infestation reduction was achieved at 25g/m^3 for the smoke of *A. herba alba* and at 35g/m^3 for *P. harmal*a smoke (table 1). Figures 2 and 3 show the results obtained for each plant we can also note that the decrease in the rate of infection by *Penicillium* spp increases with increasing doses for both plants.

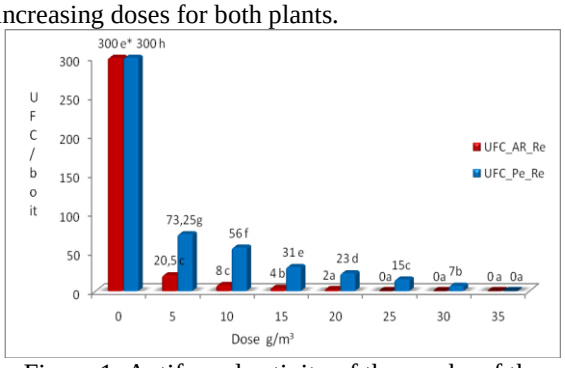

Figure 1: Antifungal activity of the smoke of the aerial parts of *Artemisia herba alba* and seeds of *Peganum harmala* burned using electric stove on the viability of spores of *Penicillium* spp.

** : For each concentration figures followed by the same letter at the histograms are not significantly*

different according to the Newman-Keuls test (P ≤ 5%).

Table 1: Mortality percentage of *Penicillium* spp spores using the fumigation of the aerial parts of *Artemisia herba alba* and seeds *Peganum harmala*.

Concentration (g / m^3)	*Artemisia herba alba*	*Peganum harmala*
0 (Control)	0 e*	0h
5	93,12d	75,58g
10	97,13c	81,33f
15	98,62b	89,67 e
20	**99,3a**	92,33d
25	100a	95c
30	100a	97,67b
35	100a	**100a**

** : Numbers followed by the same letter in the same column are not significantly different according to the Newman-Keuls test (P ≤5%).*

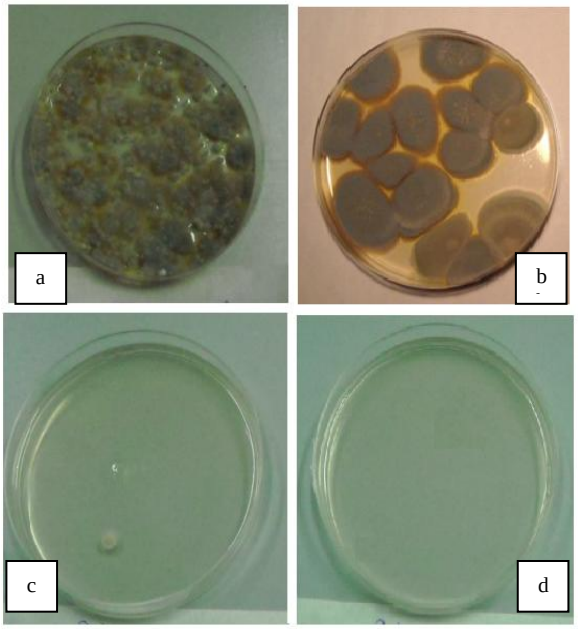

Figure 2: Results of trapping *Penicillium* spp spores after fumigation with the aerial parts of *Artemisia herba alba* smoke at the doses of 0 (a), 5 (b), 15 (c) and 20 g/m^3 (d).

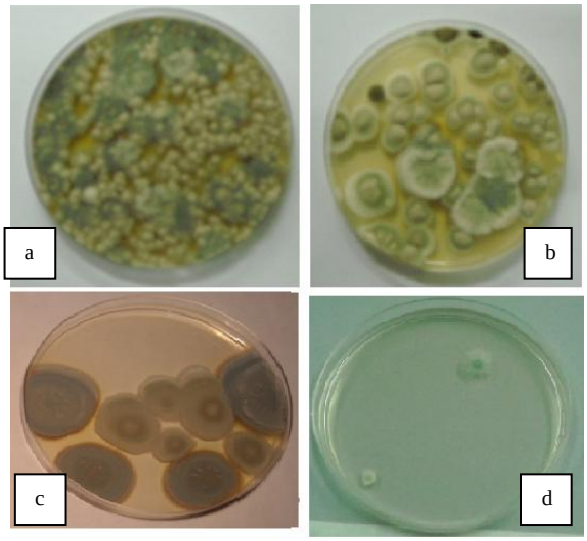

Figure 3. Results of trapping *Penicillium* spp spores after fumigation with *Peganum harmala* seeds at the doses of 0 (a), 5 (b), 20 (c) and 25 g/m^3 (d).

The results of the antifungal activity of the product Deccofenato pot are presented in table 2 and figure 4. This product showed significant efficacy against *Penicillium* spp dose of 1g/m^3. No fungus colony could grow.

Table 2: Results disinfection of cold rooms of Souss Agrumar station by the product Deccofenato pot

Cold storage room N°	*Penicillium* spp (UFC/Petri dishes)		Percentage of infestation
	Before treatment	After treatment	
7	300	0	100%
8	55	0	100%
9	300	0	100%
12	200	0	100%

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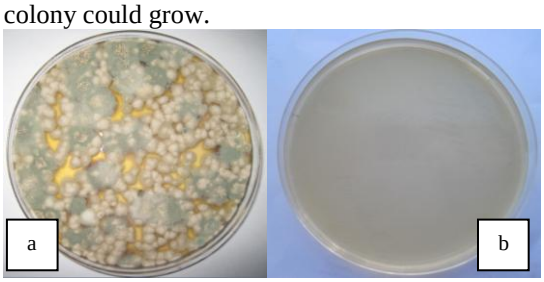

Figure 4: Results trapping spores *Penicillium* spp in the refrigeration room # 9 disinfected by Deccofenato pot control (a) and box room after treatment (b).

Complete inhibition was obtained with the aerial parts of *Artemisia herba alba* and *P. harmala*. This efficiency is probably due to the *Artemisia herba alba* essential oils. Several authors have demonstrated the efficacy of the essential oil of *Artemisia herba alba* against mycelial growth of plant pathogenic fungi;, on the fungus Fusarium *oxysporum* **Kolai et al.** [8], *Aspergillus Niger* , *Penicillium digitatum* and *Penicillium expansum* **Bencheqroun et al**. [9]. The seeds of the *P. harmala* were studied for their antibacterial [7] insecticide [10; 11] and nematicide [12]. The oil extracted from the seeds of *Peganum harmala* has already been studied by **Hassani Idrissi and Elhadek** [13] the results reveal that the oil contains the alkaloids beta carbolines as harmine and harmaline. In scientific investigations **Ameziane et al.** [14] showed that the *P. harmala* seeds used as powder showed total inhibition of mycelial growth of three fungi *P. digitatum* , *P. italicum* and *G. candidum*. It was also reported that the most volatile and antifungal vapors reduce spore germination inducing the death of fungi tested [15]. In a recent study, **Khlifi et al.** [16] reported that methanol extracts of *Artemisia herba alba*, *Ruta chalpensis* and *Peganum harmala* showed anti- oxidant activity, Cancer and anti-inflammatory activities.

IV. CONCLUSIONS

It appears from this study that the aromatic and medicinal plants used as fumigant have strong antifungal activity against *Penicillium* spp. Thus, the smoke of the aerial parts of *Artemisia herba alba* and seeds of *Peganum harmala* proved inhibiting the viability of spores of *Penicillium* spp at the doses of 25 and 35 g/m^3 respectively. At these doses the plants were effective as the pesticide Deccofenato pot used at 1g/m^3 in the cold storage room of citrus.

REFERENCES

[1] Holmes GJ, Eckert JW, Pitt JI, *Revised description of* Penicillium ulaiens *and its role as a pathogen of citrus fruit.* Phytopathology 84, 719-727. 1994

[2] Cabras (P.), Schirras (M.), Pirisi (F.M.), Garau (V.L.), Angioni (A.). *Factors affecting imazalil and thiabendazole uptake and persistence in Citrus fruits following dip treatments*. - J. Agric. Food Chem. 47(8): 3352-3354. 1999.

[3] Ben-Yehoshua S., Shapiro B., Kim J. J., Sharouni J., Carmeli S. and Kashman Y.,. *Resistance of citrus fruit to pathogens and its enhancement by curing*. Proc. Int. Soc. Citriculture 3: 1371-1379. 1988

[4] Boubaker H., B. Saadi, E. H. Boudyach and A. A. Benaoumar. 2009. *Sensitivity of* Penicillium digitatum *and* P. italicum *to Imazalil and Thiabendazole in Morocco*. Plant Pathology Journal, 8(4): 152-158

[5] Boubaker; B. Saadi ; E.H. Boudyach ; A. Ait Benaou . 2008. *Resistance of* Verticillium theobromae *to Benzimidazole Fungicides in Morocco*. Journal of Applied Sciences, V8 (21).

[6] Suwalsky M., Rodríguez C., Villena F., Aguilar F., Sotomayor C.P. - *The pesticide hexachlorobenzene induces alterations in the human erythrocyte membrane*. - Pestic. Biochem. Physiol., 65(3), 205-214. 1999

[7] Bellakhdar J, *La pharmacopée marocaine traditionnelle. Médecine arabe ancienne et savoirs populaires*, Paris - Rabat, Ibis Press - Eds Le Fennec, 764 p1997

[8] Kolai N., F. Saiah. et A. Boudia,**.** *Effet inhibiteur in vitro de l'huile essentielle d'*Artémisia herba alba *sur deux souches de* Fusarium oxysporum *f. sp*.radicis-lycopersici. Algerian journal of aride environnement vol. 2, n°1, Juin 2012 : 71-76. 2012

[9] Bencheqroun H., M. Ghanmi, B., Satrani, A., Aafi et A., Chaouch.,. *Activité antimicrobienne des huiles essentielles d'*Artemisia mesatlantica, *plante endémique du Maroc. Antimicrobial activity of the essential oil of an endemic plant in Morocco,* Artemisia mesatlantica. Bulletin de la Société Royale des Sciences de Liège, Vol. 81, 2012 p. 4 – 21. 2012

[10] Idrissi Hassani L.M., Ould Ahmedou M.A., Chihrane J., Bouaichi A. *Effets d'une alimentation en* Peganum harmala (Zygophyllaceae) *sur la survie et le développement ovarien du criquet pèlerin* Schistocerca gregaria *Forskål (Orthoptera, Acrididae*) Ethnopharmacologia, , 23, 26-41. 1998.

[11] Idrissi Hassani L.M., Ould Ahmedou M.L., Mayad E.H., Bouaichi A. *Pouvoir insecticide de* Peganum harmala : *effets de l'huile et des extraits de feuilles.* Biologie et santé. 2001.

[12] Mayad E.H, Z. Ferji, B. Chebli et L.M Idrissi Hassani. *Étude* in vitro *du potentiel nématicide de quelques extraits de plantes médicinales*. Reviews in Biology and Biotechnology. Vol .5, No 2. pp. 37-40. 2006.

[13] Idrissi Hassani L.M., EL Hadeck M.. *Analyse de la composition de l'huile de* Peganum harmala L. (Zygophyllacées). Acta Botanica Gallica, , 146 (4), 355- 361. 2000

[14] Ameziane N, H. Boubaker, E. H. Boudyach, F. Msanda, A. Jilal and A. Ait Benaoumar. *Antifungal activity of Moroccan plants*

against citrus fruit pathogens.. Agron. Sustain. Dev. 27: 273-277. 2007.

[15] Sholberg P. L. *Fumigation of fruit with short-chain organic acids to reduce the potential of postharvest decay*. Plant Disease. 82: 689-693. 1998.

[16] Khlifi D, Sghaier RM, Amouri S, Laouini D, Hamdi M, Bouajila J. *Composition and anti-oxidant, anti-cancer and anti-inflammatory activities of* Artemisia herba-alba, Ruta chalpensis L. *and* Peganum harmala L. Food Chem Toxicol. 55:202-208. 2013.