PRELIMINARY RESEARCH REGARDING OREGANO ESSENTIAL OIL ANTIMYCOTIC ACTIVITY AGAINST STORED FUNGI

Roxana Dudoiu[1], Carmen Lupu[1], Mariana Popescu[2], Elena Radu[2]*

[1]Research Development Institute for Plant Protection, Bucharest
[2]The National Institute for Research & Development in Chemistry and Petrochemistry

**correspondence address:*
Research Development Institute for Plant Protection
8 Ion Ionescu de la Brad
013813, Bucharest, ROMANIA
Tel: 004-021-2693231
Fax: 004-021-2693239
E mail: roxyanna_21@yahoo.com

Abstract: Essential oils (EOs) have been long recognized for their antibacterial, antifungal and antiviral properties. Worldwide, at the scientific community level there have been discovered a series of plant bioactive eco-friendly principles, and were performed a multitude of researches regarding the potential of essentials oils and mixture of organic volatile compounds, as alternative insecticides and antimicrobial fumigants for a sustainable agricultural production. The increased interest in alternative natural substances is driving the research community to find new uses and applications of these substances. EOs and their components show promising activities against many pathogens and spoilage microorganisms when tested in vitro. The use of combinations of EOs and their isolated components are thus new approaches to increase the efficacy of EOs in microorganisms' control, taking advantage of their synergistic and additive effects. Among promising alternative methods to control food spoilage much attention is being paid to the use of essential oils (EOs), and lately also to their activity in vapor phase. This paper presents preliminary research regarding the oregano essential oil antimycotic activity towards a series of stored fungi.

Key words: *oregano oil, storage fungi, antimicrobial activity.*

INTRODUCTION

Essential oils (EOs), aromatic oily liquids of plant origin, have been used for their diverse biological activities by humans since Middle Ages (Bakkali, et. al., 2008). In the food industry, after several decades of the synthetic antimicrobials dominion, essential oils are once again being proposed as food preservatives (Tiwari et al., 2009). EOs are volatile substances with an oily consistency typically produced by plants. They can be liquid at room temperature and showing different colors ranging from pale yellow to emerald green and from blue to dark brownish red (Balz, 1999). They are synthesized by all plant organs and are stored in secretary cells, cavities, canals, epidermis cells or glandular trichomes (Bakkali et al, 2008). Several techniques can be used to extract EOs from different parts of the aromatic plants, including water or steam distillation, solvent extraction, expression under pressure, supercritical fluid and subcritical water extractions.

The *in vitro* antimicrobial activity of EOs has been studied against a number of microorganisms, usually using direct-contact antimicrobial assays, such as different types of diffusion or dilution methods, as reviewed by some literatures (Holley & Patel, 2005; Janisiewicz & Korsten, 2002; Tripathi & Dubey, 2004; Burt, 2004).

The aim of this study is to evaluate the efficacy of the oregano essential oil in vapor phase against several storage pathogens belonging to *Fusarium, Aspergillus, Penicillium* and *Phythophtora* genera.

MATERIALS AND METHODS

Essential oil The essential oil of oregano was purchased from Cozak Plant and stored at +4°C in a refrigerator until analysis. There was a performed a Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of the oregano essential oil in order to determine the percentage of the main volatile compounds. The analysis was carried out using with 7000 Triple Quad GC/MS Agilent. A DB-WAX capillary column of 30 m x 0.25 mm and 0.25 in film thickness was used. Helium was used as a carrier gas (1.4 ml/min). The column was temperature programmed as follows: 2 min at 70°C; then raises with 10°C/min to 220°C and held for 10 min. The injector and detector temperatures were to 220 and 250°C, respectively. Injection was carried out automatic mode. Peak areas and retention times were measured by Electronic Integration.

Microorganisms The screening of the antifungal activity was performed against five strains of fungi isolated from cereals samples, taken from a warehouse in Prahova County. The fungi taken into analysis consisted in two strains of *Fusarium tricinctum* and *F. graminearum*, one strain of *Penicillium expansum*, a strain of *Aspergillus versicolor* and one strain of *Phythophtora* sp. (figure 1). Stock cultures of fungal strains were grown on potato-dextrose-agar medium at 27°C for 7 days before the experiment.

Figure 1. Test fungi strains on PDA medium

An amount of 15ml of sterile PDA medium was poured into 10cm diameter Petri dishes. After solidifying, plates were kept in an inverted position; a sterilized filter paper disk of 10mm diameter was placed in the center of each plate's lid and 0, 2, 4, 8, 16, 32 and 64µl of pure oregano essential oil were added to the paper. At the same time, fungal mycelia-disks (diameter of 5mm) prepared from growing margin of each isolate were placed in the center of PDA plates (figure 2). Control plates contained equivalent amounts of distilled water. Plates were tightly sealed with parafilm and incubated at 27°C for 14 days. Diameters of the growing colonies were measured weekly.

Figure 2. Inoculation aspects

RESULTS AND DISCUSSIONS

Identification of the compounds was achieved by comparing retention times and mass spectra with those of the NIST database. As presented in figure 3, the oregano oil analyzed had a high

percentage of Carvacol – 69,6%, followed by 15,73% o-Cymene, 4,86% Caryophyllene, 2,12% Terpinen, 1,13% Limonene and 1,21% α-Pinene.

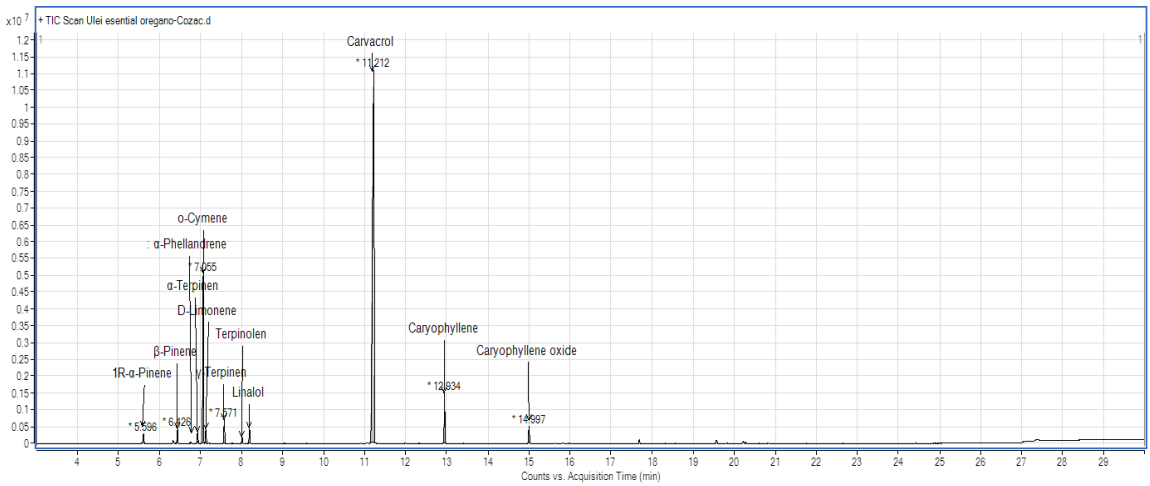

Figure 3. Oregano oil chromatogram

All five fungal strains were susceptible to the oregano essential oil vapors; the most susceptible ones were *Fusarium* and *Phythophtora* strains. The most resistant strains were *Penicillium expansum* and *Aspergillus versicolor* (figure 4).

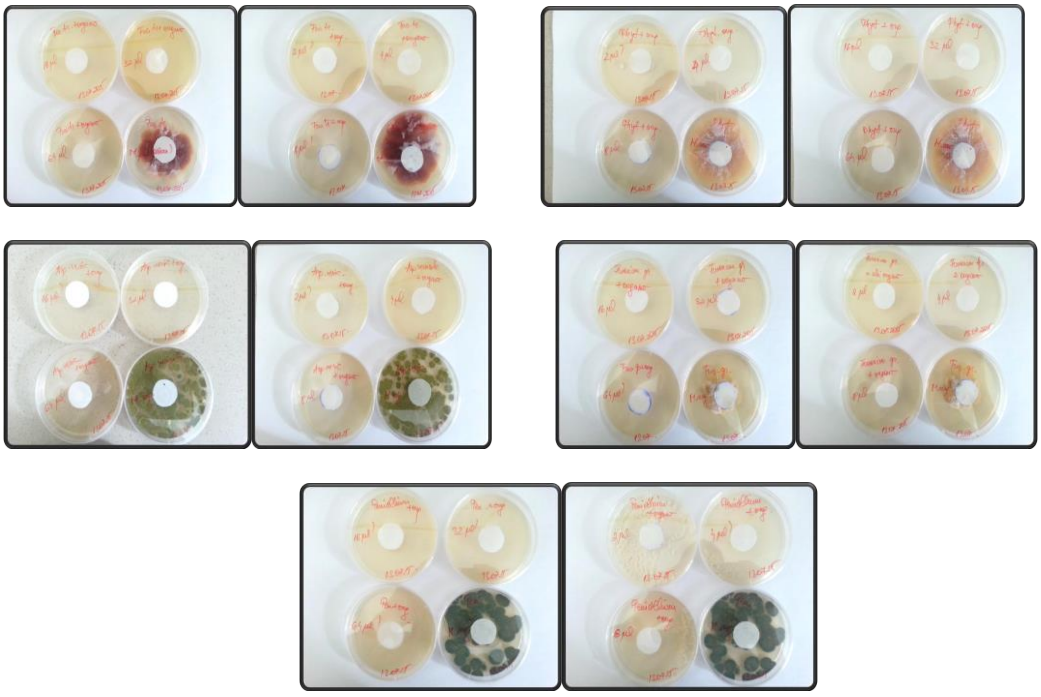

Figure 4. Tested fungi inhibited by different oregano EO concentrations compared with negative control

Analyzing the mycelia growth from each Petri dish, it can be observed that after 7 days, the essential oil of oregano applied in 6 different concentrations, inhibited completely the development of *Fusarium tricinctum*, *F. graminearum*, and *Phythophtora* sp. Regarding *Aspergillus versicolor* and *Penicillium expansum*, the vapors of oregano essential oil exerted

also an inhibitory activity of 90 percent, the vegetative growth of both strains being very weak, with only a few colonies, with no pigment.

Due to the disk filter paper method used, it was demonstrated by the growth inhibition of the microorganisms, which was uniform, and no "inhibition zones" indicating uneven concentration of active constituents were observed.

The experiments will be continued in order to establish a method by which will be able to determine if the essential oils have also activity to suppress the mycotoxins' secretion.

CONCLUSIONS

The oregano essential oil tested in all 6 concentrations, manifested a strong antifungal activity by totally inhibiting the growth of interest pathogen strains.

ACKNOWLEDGEMENTS

The study is founded by UEFISCDI, PCCA2013, Contract no. 156/2014, Ecological products based on diatomaceous earth and essential oils for the residues and contaminants reduction from the food chain – PEDIOL.

REFERENCES

BAKKALI, F., AVERBECK, S., AVERBECK, D., WAOMAR, M. (2008). Biological effects of essential oils -A review. Food and Chemical Toxicology, 46 (2): 446-475.

BALZ, R. (1999). The Healing Power of Essential Oils, 1st ed., Lotus Press: Twin Lakes, WI, USA, 27-80.

BURT, S. (2004). Essential oils: their antibacterial properties and potential applications in foods – a review**.** International Journal of Food Microbiology, 94, 223-253.

HOLLEY, R. A., PATEL, D. (2005). Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiology, 22 (4): 273-292.

JANISIEWICZ, W.J., KORSTEN, L. (2002) Biological control of postharvest diseases of fruits. Annual Review of Phytopathology, 40:411-444

TRIPATHI P, DUBEY NK. 2004. Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Postharvest Biology & Technology, 32, 235-245.

TIWARI BK, VALDRAMIDIS VP, O'DONNELL CP, MUTHUKUMARAPPAN K, BOURKE P, CULLEN PJ. (2009). Application of natural antimicrobials for food preservation. Journal of Agricultural and Food Chemistry, 57:5987–6000.