Romanian Journal for Plant Protection, Vol. VIII, 2015
ISSN 2248 – 129X; ISSN-L 2248 – 129X

METHOD FOR FUNGISTATIC ACTIVITY DETERMINATION OF *THYMUS VULGARIS* ESSENTIAL OIL

Viorel Fătu[1,2*], *Carmen Lupu*[1]

[1] Research and Development Institute for Plant Protection, Bucharest
[2] University of Agronomic Science and Veterinary Medicine, Bucharest

[*] *correspondence address*
Research and Development Institute for Plant Protection
8 Ion Ionescu de la Brad
013813, Bucharest, Romania
Phone: + 40 21269 32 31
Fax: + 40 21269 32 39
e-mail: fatu_viorel@yahoo.com

Abstract: Several experiments of antimicrobial activity assessment of the essential oils were carried out using classical microbiological methods, involving the use of media with high water content. Because of the hydrophobic properties of essential oils, their contact with the target pathogens is limited by water films. From previous experience, it was demonstrated that the use of essential oils in vapour phase is more effective than in the dissolved form in the medium by means of an emulsifier. The method aims to assess the inhibitory activity of essential oils on cereals pathogens by the action of released vapor. The minimum inhibitory concentration (MIC) is calculated by assessing a biochemical compound found in the stored seeds (e.g. starch) which serves as energy substrate in pathogen metabolism. Using this method has determined the minimum inhibitor volume between 1-5µl thyme essential oil extract, evaporated in a liter of air for *Aspergillus* spp.

Key words: *essential oil, antimicrobial, vapour phase, substrate*

INTRODUCTION

Grain storage is a technology sequence in the agrifood chain which connects the harvest time with the beginning of processing. This sequence may be conducted on a ranging time from a few weeks to several years. The first condition for grain storage is the percentage of moisture content should not exceed 12%. In some cases the water content of the stored grain mass may increase compared with the moment of the storage beginning. These increase could be caused by meteorological events, seeds own physiological activity or insects and pathogens metabolism. Essential oils use in food preservation begins to receive increased importance due to non-acquiring drug resistance by deposit pathogens and their low toxicity (Fisher, 2008; Shaaban et al., 2012). The composition of plant essential oils extracts is the result of a long process of evolution due to the pathogen and plant coexistence in the competition for resources and survival. Current assessment of the antifungal activity of an essential oil is made by comparative measurements of growth rates between the control sample and the test sample after growing on an agreed culture medium. This assessment is usually unilateral: an extract is tested together with a pathogen. But in grain deposits is formed a competitive consortium between pathogens, and the inhibition of one pathogen could lead to stimulation of another pathogen. To avoid this inconvenience it have resorted to making a bioassay assessment of fungistatic activity using an essential oil (*Thymus vulgaris*) and samples of corn grain (*Zea mays*) from a cereal storehouse with unaltered microbial load. The aim of this method is to assess the inhibitory activity of essential oils on cereals pathogens by the action of released vapor.

MATERIALS AND METHODS

Corn samples were collected from S.C. AGROTEHNIC Păulești deposit from areas susceptible to pathogenic infections (cold corners and humid floors). Samples were mixed together, milled and brought to 25% humidity from 10% to create favorable development conditions for existing pathogens in grain mass. After moistening, initial starch concentration (Ci) was evaluated by extraction in 90% dimethyl sulfoxide (DMSO) and polarimetric determination with P3001 RS device (Garcia & Wolf, 1971). Seven concentrations of thyme essential oil extract deposited on cellulose support were tested (1, 2, 5, 10, 20, 50 and 100µl/L air) inside 3,2L containers loaded with 300g milled corn (25% moisture). After seven days of incubation at 30°C, the samples showed no infection symptoms, except the control (Figure 1).

Figure 1. 300g milled corn with their own microbial load after seven days of incubation at 30°C, left -100µl/L air *Thymus vulgaris* volatile oil, right – control.

The incubation was extended to two months at room temperature. At the end of the incubation period, the starch concentration was determined according to the following protocol: grind samples for 3 - 5 minutes > weigh 1 gram (W) > the weighed sample is added in 20 ml (V1) DMSO (90%) > shake sample for 24 hours > mix 10 ml extract (V2) with 45 ml (V3) DMSO (90%) > read the rotation angle using the polarimeter > starch concentration is calculated by the following formula:

$$\% \text{ starch} = \frac{(\alpha) \times 100(V1) \times (V3/V2)}{[\alpha]^{25}_{546} \times P \times W \times (100 - \%\text{moisture})}$$

Where: (α) – sample observed optical rotation angle, in degrees;
V_1 – 20 ml DMSO (90%);
V_2 - 45 ml DMSO (90%);
$[\alpha]^{25}_{546}$ - = 220° = starch specific rotation angle in 90% DMSO ;
P - Optical path length, in dm;
W – Weight of milled corn (1g).

For the determination of minimum inhibitory concentration, the next formula was used:

$$\text{Growth inhibition (\%)} = \frac{(C_t - C_m) \times 100}{C_i - C_m}$$

Where: C_t – Starch concentration in the presence of the inhibitor;
C_m – Starch concentration in the absence of inhibitor;
C_i – Starch concentration before incubation.

RESULTS AND DISCUSSIONS

After starch percent determination of test and control samples (Figure 2), the volume of 2µl/L air of thyme essential oil extract was considered the minimum inhibitory concentration (MIC).

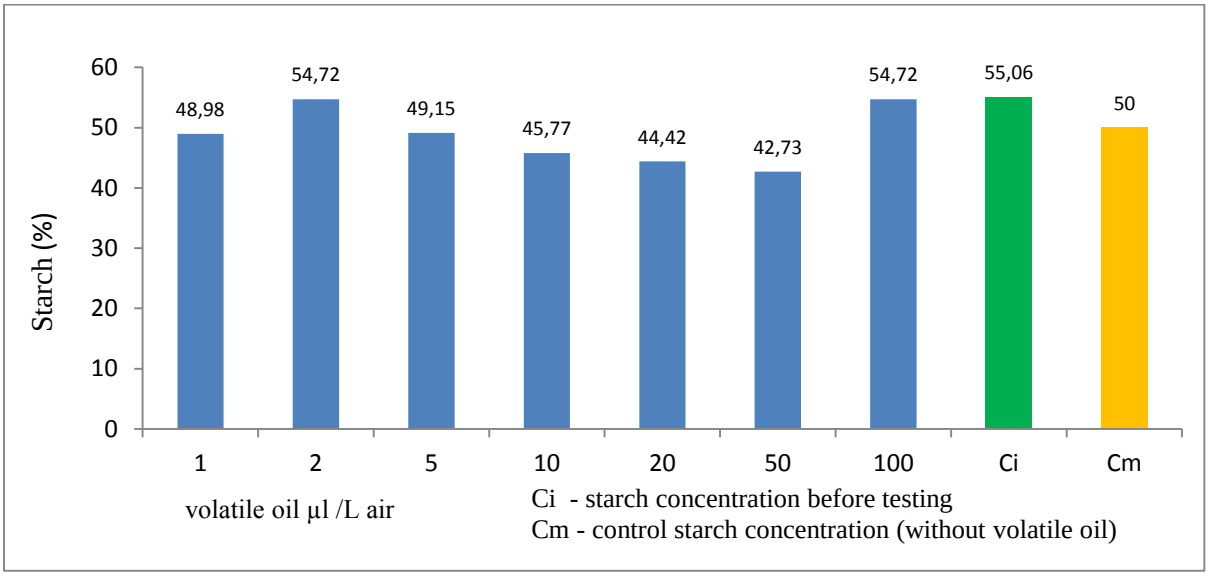

Figure 2. The starch percent in test and control samples after two months incubation

This concentration value presents practical importance because there are required only 2ml of thyme essential oil extract to protect $1m^3$ airtight cereal storage. In a parallel experiment, the fungistatic activity of essential oils of thyme was tested (100µl oil /L air) for a period of three months at room temperature (Figure 3).

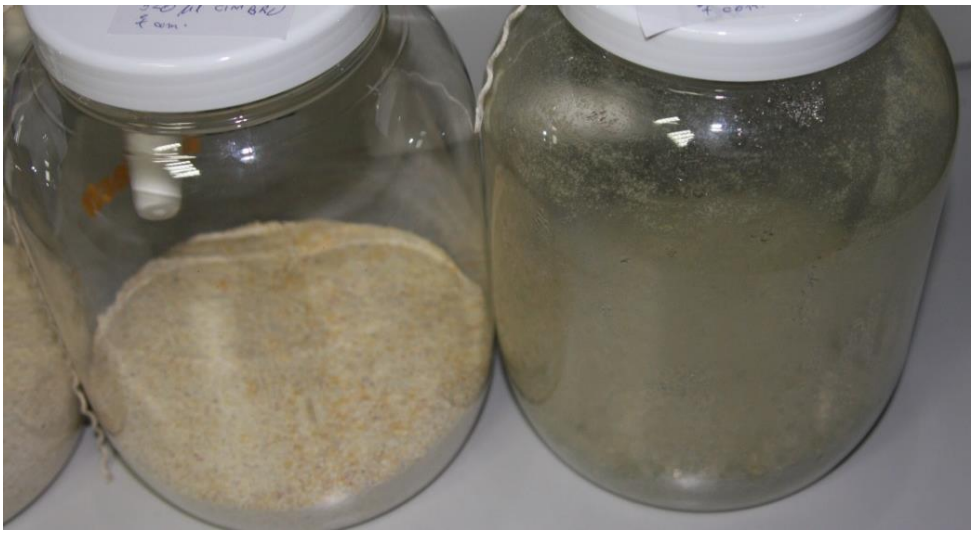

Figure 3. 300g milled corn with their own microbial load after after 3 months incubation at room temperature, left -100µl/L air *Thymus vulgaris* oil, right – control.

During this period, the starch content of the control sample decreased from value of 55.06% to 39.18%, and the water content increased from 25% to 47.14%. The sample which contains 100µl/L air thyme essential oil extract lost 4.22 percent of starch and won 2.86 percent of water. Using the proposed fungistatic assessment formula, it can be obtained simultaneously the inhibitory activity and the stimulating effect (Figure 4).

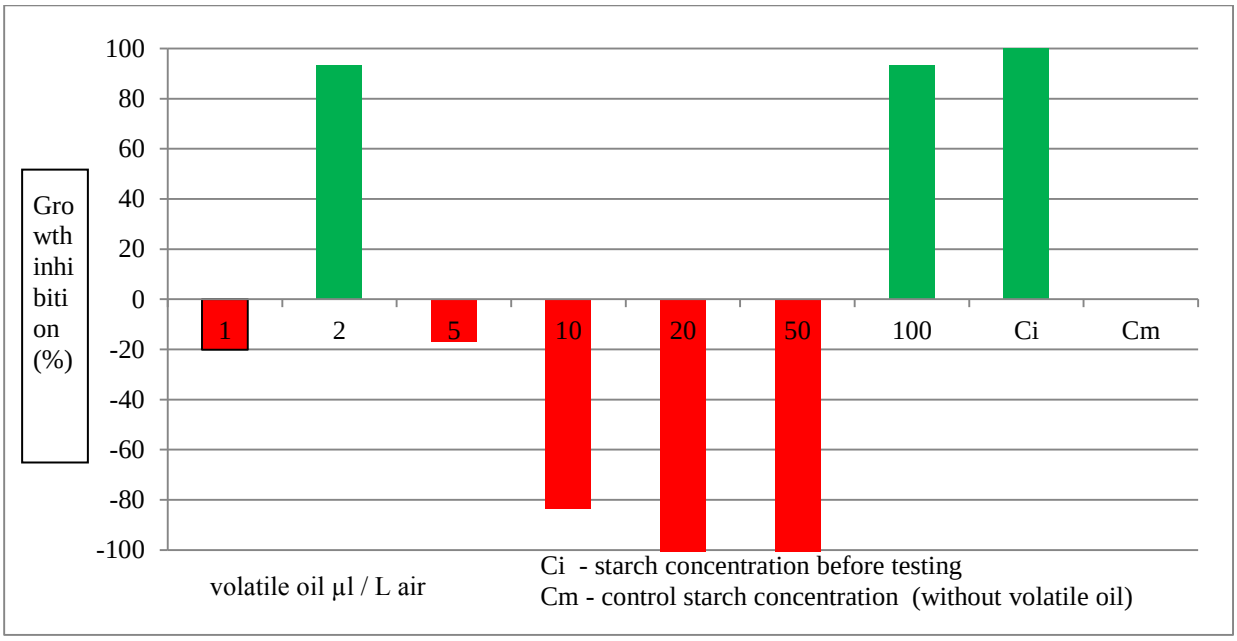

Figure 4. Growth inhibition of microbial load at various concentrations of *Thymus vulgaris* essential oil.

According to the rates of water and starch change, it can be concluded that there is a negative correlation between the starch consumption by pathogens of and water generated from metabolic reactions (Figure 5).

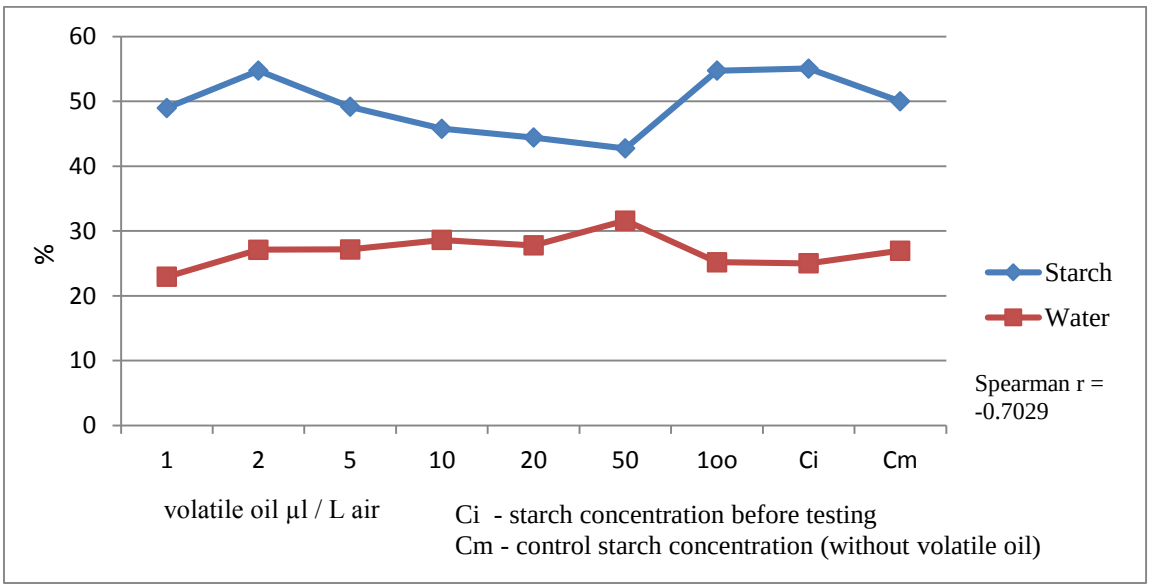

Figure 5. Correlation between the starch and water content

CONCLUSIONS

The use of *Thymus vulgaris* volatile oil extract in stored grain protection it might be an environmentally friendly alternative to classical methods of storage pathogens control. But a dosage which has a deviation from the MIC with a few microliters can produce inhibition of a pathogen that is in an antagonism relationship with another pathogen, resulting in greater losses of biochemical compounds than in the absence of volatile oil. The next effective concentration of volatile oil in protecting stored grain is situated around 100µl/L air. This concentration is not useful in practical terms of quality and economy, as it alters the olfactory properties of stored grain and is an expensive solution. The validation of the experiment and method is made by calculating the correlation between the amount of consumed starch and the amount of resulting water at the end of the incubation period.

ACKNOWLEDGEMENTS

The study was funded by UEFISCDI PCCA 2013 Contract no. 156/2014 "Produse ecologice pe bază de diatomită și uleiuri esențiale pentru diminuarea reziduurilor și contaminanților din lanțul alimentar" – PEDIOL.

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