ANTIFUNGAL ACTION OF THE THYME (*THYMUS VULGARIS* L.) OIL ON SOME MYCOTOXYCOGENOUS FUNGI *ASPERGILLUS FLAVUS* LINK. AND *FUSARIUM GRAMINEARUM* SCHW. L

Daniela Raluca[1]HERȚEG, Otilia COTUNA [1], Georgeta POP [1]

[1]Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Romania
Corresponding author: e-mail: otiliacotuna@yahoo.com

Abstract. *Mycotoxicogenous fungi Fusarium graminearum and Aspergillus flavus are producing mycotoxins in the cereal grains, that through the nutritive value and the increase of the toxicity of the plant products, these cannot guarantee health and they will not provide the security of the life. From this reason these two fungi are in the attention of the researchers and cereals cultivators from the worldwide. The antifungal action of the thyme essential oil on these two fungi was tested through diffusion in the culture medium Sabouraud with chloramfenicol in Petri plates in three doses: 1 µl, 5 µl and 10 µl. The obtained results in the framework of this research are evidencing the great capacity the thyme oil on the inhibition of the development of the two fungi. On the media treated with essential oil in different doses the number of the colonies was very low (between 1 and 7) in comparison with the control tester were growth between 31 and 38 mycelian colonies. Inhibitory capacity of the thyme on the two mycotoxicogenous fungi was proved to be very good, the growth speed being very low in all variants in comparison with the non-treated testers. The present researches show that in the antifungal activity are implied the fenolic compounds carvacrole and thymole. The very good antifungal capacity of the thyme volatile oil recommends it for the use in the control of some storage pathogens that cannot be kept under control through other methods.*

Key words: *Aspergillus flavus, Fusarium graminearum*, essential oil, thyme, mycotoxins.

INTRODUCTION

Antifungal action of the thyme essential oil presents great interest in the control of some pathogens from the cereal crops, mainly for fungi from the genus *Aspergillus* and *Fusarium.* The main compounds from the thyme oil that have proved to have great antifungal activity are the following: thymol, P - cymene, Y - terpinene, carvacrole. MOGHTADER M. *ET AL*. (2012) show that the antifungal activity of the thyme oil against the *Aspergillus niger* fungus is stronger than in the case of streptomycin and gentamycine antibiotics. The great inhibition percentage of the fungi growth can be assumed to the thymol that is the main compound of the thyme oil. There was noticed that the antifungal effect depends a lot by the oil concentration, by the target pathogen and by the action of the natural compounds.

In the last years the antifungal capacity of the thyme oil was tested on the pathogenic fungi *Aspergillus flavus* and *Fusarium graminearum.* With the thyme essential oil there were tested other essential oils too, respectively fennel oil (*Foeniculum vulgare* Mill.), mint oil (*Mentha piperita* L.), ginger oil (*Zingibir officinale* Roscoe) - SILVA FERNANDA C. DA *ET AL*., 2012. The use of the plants essential oils is attractive at this moment because they can replace the chemical treatments (REDDY *ET AL*., 2010a).

Aspergillus flavus is a major producer of aflatoxins in field and in store. The prevention of contamination with mycotoxins regards the use of strategies that doesn't let the

pathogen to develop in the cereal crops and later in store. Many researches confirm the antifungal effect of the thyme oil on the fungus *Aspergillus flavus*. The experimental results obtained show at concentrations of 200 ppm the fungus growth was diminished with 81% and over 500 ppm was completely inhibited (SOLIMAN & BADEA, 2002; RAZZAGHI - ABYANEH *ET AL*., 2009; NGUEFACK *ET AL*., 2004).

In a research from 2004, Zambonelli *et al*. show that thymole actions on the fungal cell producing changes at the level of mitochondria and endoplasmic reticulum together with the accumulation of the lipid drops. Other authors have noticed in the case of the fungus *Aspergillus niger* the collapse of the hyphae, broke of the plasmatic membrane, damaging of the mitochondria (RASOOLI *ET AL*., 2006). Due to the great antifungal activity, the thyme oil is intense studied having in view the application in practice (RAHIMIFARD N. ET AL., 2008).

Fusarium graminearum is a fungus that appears in the cereal crops and is spreading when are meet the optimal development conditions (high humidity and low temperatures). The fungus is often found both inside and outside the cereal grain (KOCIĆ-TANACKOV D. S., DIMIĆ R. G., 2013). Its presence in the cereal mass is highly correlated with the formation of the mycotoxins (DON, zearalenone, nivalenole). This pathogen is causing important loses in cereal crops all over the world (STARKEY *ET AL*., 2007). The losses appear both in the case of the yield per hectare and in its quality due to the contamination with mycotoxins (ZHANG H. *et al*., 2012). In Romania, fusarium root is a disease with great economic importance, mainly in the years when the climatic conditions are favourable to the fungus growth and spread.

At worldwide level are developing alternatives to the chemical fungicides used nowadays because in the case of many of them *Fusarium graminearum* have developed a great resistance. Practically there is no variety resistant to the attack of this pathogen. Other aspects are the problems created by fungicides residues with high toxicity for the human health and for the environment (LIU N. *ET AL*., 2013; SUN H. Y. *ET AL*., 2014).

As in the case of the fungus *Aspergillus flavus* the studies are showing that the thymole has proved to be very efficient also in the inhibition of the fungus *Fusarium graminearum*. The antifungal mechanism of the thymole on the plant pathogen fungi isn't yet fully elucidated. Recent researches highlight the capacity of this component of the thyme oil in the inhibition of the conidia formation and the hyphae growth in the case of *F. graminearum* determining the deterioration of the cell membrane. The thymole compound acts on fungi through the initiation of an osmotic stress. The typical indicator for the osmotic stress in fungi is the accumulation of glycerol. The strong antifungal action of the thymole recommends it in the control the fusarium root disease in cereals (TAO GAO *ET AL*., 2016).

Fusarium graminearum and *Aspergillus flavus* are pathogens difficult to be controlled; respectively the control of the first one is a priority at worldwide level (MORCIA *ET AL*., 2011; BECHER R. *ET AL*., 2013).

In this research the antifungal action of the thyme essential oil on the two fungi was tested by diffusion in the culture medium in different doses. The obtained results are highlighting the very good capacity of inhibition of the thyme oil on the growth of the two pathogens.

MATERIAL AND METHOD

The isolation of the *Fusarium graminearum* and *Aspergillus flavus* was done from the surface of maize grains, where the mycelia where developed during the period when they have been kept in the wet chamber in incubator for 7 days at a temperature of 23±2 °C. The culture

medium used was Sabouraud Agar 4% with chloramphenicol. This medium has a high concentration of glucose, favourable to the fungi growth (stable from osmotic point of view), while most of the bacteria doesn't tolerate high sugar concentration. Also, the reduced value of the pH is optimal for fungi, but not for many bacteria.

The antifungal capacity of the thyme oil was tested by diffusion in the semisolid Sabouraud culture medium in Petri plates. The essential oil was applied by incorporation in niches realised on the surface of the environment in three doses 1µl, 5 µl and 10 µl. The inoculation of the culture media treated with essential oil was done by the placing of mycelium fragments in several points using an inoculation needle previously sterilized in flame, the after the solidification of the culture medium. The incubation of the Petri plates was done at the temperature of 25°C for 5 days. The Petri plates without essential oil were considered control variants. There were obtained eight variants: 4 for the fungus *Aspergillus flavus* and 4 for *Fusarium graminearum*.

The growing speed of the mycelium was calculated with the following formula:

$$V = \frac{dc}{t}$$

where V = mycelium growth speed; dc = colony diameter; t = time in hours.

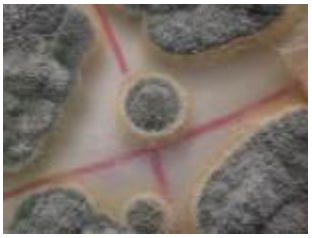
Inhibition rate = {(C-T)/C} x 100

where: C is the diameter of the control colony and T is the diameter of the colony growth on the treated medium.

The thyme essential oil tested in this experience was obtained in the laboratory of the discipline Crop Science. The thyme was cultivated in the Didactic Station of Banat's University of the Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara. The vegetal material used for the oil extraction was harvested at the flowering phase, when the oils accumulation is intense. The essential oil was obtained by hydro-distillation.

RESULTS AND DISCUTIONS

The results obtained in this research are highlighting the great capacity of inhibition of the thyme essential oil on the development of the two pathogens. In comparison with the non-treated controls, where have growth among 31 and 38 mycelia colonies (figure 1), on the cultivation media treated with essential oil in different doses, the colonies number was very small (among 1 and 7). The inhibition capacity of the thyme oil on the two mycotoxigenous fungi was proved to be very good, the growing rate being very small in all the variants in comparison with the non-treated controls.



A

B

Figure 1. Mycelia colonies developed on the culture media from the control variants: A - *Aspergillus flavus*; B - *Fusarium graminearum (Photo: Otilia Cotuna, 2016)*

Thus, in the case of the fungus *Aspergillus flavus*, in the control variant the mycelium had started to grow rapidly. In all the four inoculated spots have been formed among 4, 7, 10 and 17 mycelium islands after 5 days. The diameter of the mycelium islands varied among 9 and 36 mm. on the culture medium from the variant treated with 1 µl thyme essential oil were grown among 1 and 2 mycelium islands in all the four inoculated spots with a diameter of about 1 mm. The second variant treated with 5 µl essential oil has favoured the growth of 2 mycelium islands with a diameter of 2 mm. in two spots from the four haven't grown fungal colonies. In the third variant treated with 10 µl essential oil the fungus almost hasn't growth, there being formed only one mycelium island with a diameter of about 1 mm (table 1).

The growing rate of the mycelium in the control variant was 0.214 mm/h, and in the variants 1 and 2 of 0.0059 mm/h. The lowest growing rate was registered in the case of the third variant (0.0047 mm/h).

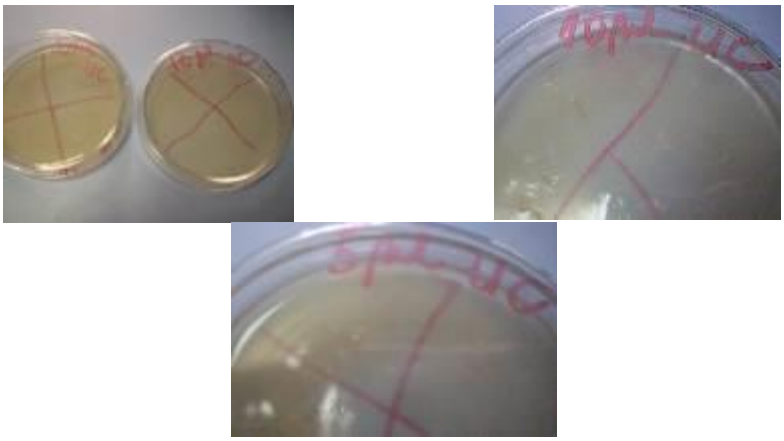


Figure 2. Mycelium colonies with very small size, developed on the culture media treated with thyme oil in different doses: A - *Aspergillus flavus*; B și C - *Fusarium graminearum (Photo: Otilia Cotuna, 2016)*

Table 1

Antifungal action of the thyme oil on the fungus *Aspergillus flavus*

Variant	**No. of formed mycelium islands**	**Colonies diametre**	**Mycelium growing rate mm/h (168 h)**
Control	7/17/ 10/ 4 Total 38	9 - 36 mm	0.214
V_1 - 1µl	2/2/2/1 7	1 mm	0.0059
V_2 - 5µl	1/1/0/0 2	2 mm	0.0059
V_3 - 10µl	1/0/0/0 1	0,8 mm	0.0047

Regarding the fungus *Fusarium graminearum*, in the control variant the mycelium was developed very fast. After 5 days inn all the four inoculated points were developed 3, 6, 10

and 12 mycelium islands. The diameter of the mycelium islands has oscillated between 8 and 45 mm. on the culture medium treated with 1 µl thyme essential oil has grown only one mycelium island with 3 mm diameter. In the second variant treated with 5 µl essential oil have grown 2 mycelium islands of 2 mm diameter. In two points from all four weren't developed colonies. In the third variant treated with 10 µl essential oil, the fungus has formed 2 mycelium colonies with the diameter of about 2 mm. the growing rate of the mycelium was 0.267 mm/h. in the variant 1 the fungus growing rate has reached 0.017 mm/h. in the variants treated with 5 and 10 µl essential oil the growing rate was 0.0059 mm/h (table 2).

Table 2

Antifungal action of the thyme oil on the fungus *Fusarium graminearum*

Variant	No. of formed mycelium islands	Colonies diametre	Mycelium growing rate mm/h (168 h)
Control	31	8 - 45 mm	0,267
V_1 - 1µl	1	3 mm	0,017
V_2 - 5µl	2	2 mm	0,0059
V_3 - 10µl	1	2 mm	0,0059

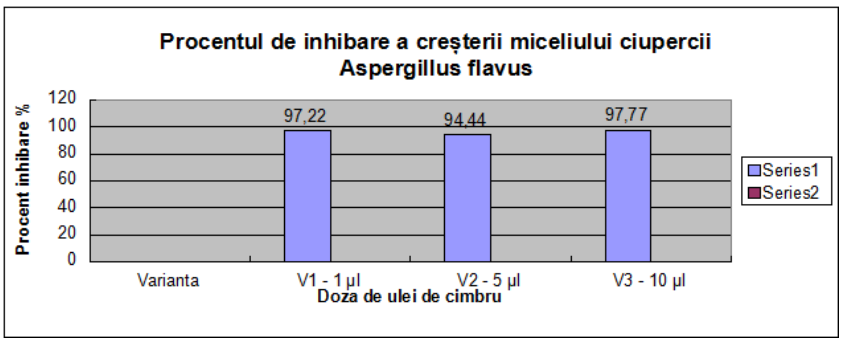


Figure 3. Inhibitor capacity of the thyme oil on the growth of the mycelium of the fungus *Aspergillus flavus*

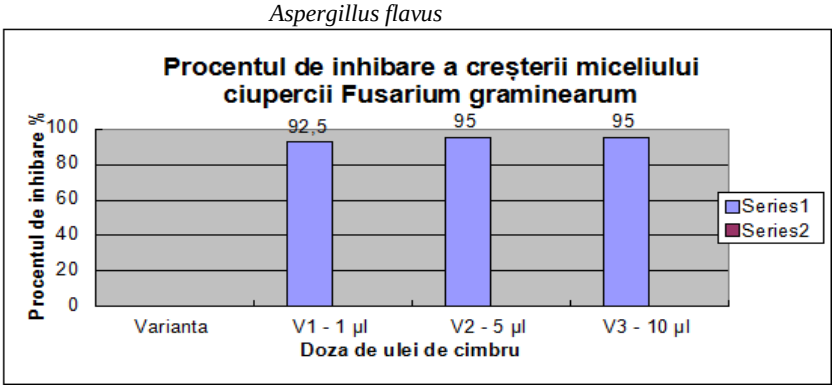


Figure 4. Inhibitor capacity of the thyme oil on the growth of the mycelium of the fungus *Fusarium graminearum*

The inhibition rate of the mycelium growth was the supposed one, passing over 90% in the case of both pathogens. Thus, the inhibition rate was comprised between 94 - 97% in the case of the fungus *Aspergillus flavus* (figure 3). In the case of *Fusarium graminearum* the inhibition rate was comprised between 92 - 95% (figure 4). The obtained results following this research are coming in the completion of the other from literature and highlight the great antifungal features of the thyme oil, that are making it attractive for the use in the control of some pathogens.

CONCLUSIONS

The thyme oil used in this research has a great inhibition capacity of the growth of the fungi *Aspergillus flavus* and *Fusarium graminearum.* In comparison with the non-treated control where have grown mycelium colonies with great diameter, on the culture media treated with essential oil in different doses the number of the developed colonies was very low and the diameter hasn't growth bigger than 3 mm.

Thus, there was noticed that on the treated media the fungi have stopped their growth, the mycelium remaining at the same size.

The inhibition rate of the mycelium growth was great, passing over 90%, thus in the treated variants the inhibition rate was comprised among 92% and 97%.

BIBLIOGRAPHY

BECHER R., MIEDANER T., WIRSEL S. R., 2013 - Biology, Diversity, and Management of FHB - Causing *Fusarium* Species in Small-Grain Cereals. In Agricultural Applications; Kempken, F., Ed.; Springer Berlin: Heidelberg, Germany; Volume 11, pp. 199–241.

GAICA I. ET AL, 2013, *The influence of fertilization on microbiological activity on degraded land after mining*, Current opinion in Biotechnology, Nr. 24

GAOTAO, HAO ZHOU, WEI ZHOU, LIANGBIN HU, JIAN CHEN, ZHIQI SHI, 2016 - The Fungicidal Activity of Thymol against *Fusarium graminearum* via Inducing Lipid Peroxidation and Disrupting Ergosterol Biosynthesis, Molecules 2016, 21, 770; doi:10.3390/molecules21060770;

GEORGETA POP, IMBREA ILINCA MERIMA, SĂRAC I., IMBREA FL.,CIOBOTARU G.V., DANCIU CORINA, SMETAN S., Antibacterial effect of Cupressus arizonica l. essential oil on different microorganisms, tested in vitro, Planta Medica , 2016; 81(S 01): S1-S381, DOI: 10.1055/s-0036-1596399

IMBREA ILINCA MERIMA, BUTNARIU MONICA, NICOLIN ALMA, IMBREA FLORIN, *Determining antioxidant capacity of extracts of Filipendula vulgaris Moench from south-western Romania*, Journal Of Food Agriculture & Environment Volume: 8, 2010, Issue: 3-4, Pages: 111 -116

IMBREA ILINCA MERIMA, MONICA PRODAN, ALMA NICOLIN, FLORIN IMBREA, MONICA BUTNARIU,2010, *Valorising Thymus glabrescens willd. From the Aninei Mountains*, Researche Journal of Agricultural Science, 42 (2)

IMBREAILINCA, ALMA NICOLIN, FLORIN IMBREA, MONICA PRODAN, MONICA BUTNARIU, Studies concerning medicinal and aromatic plants in the Minişului Valley, Researche Journal of Agricultural Science, 2010, 42 (2),

KOCIĆ-TANACKOV D. S., DIMIĆ R. G., 2013 - Fungi and mycotoxins - Food contaminants. *Hem. Ind.*; DOI:10.2298/HEMIND120927108K.

LIU N., FAN F., QIU D., JIANG L., 2013 - The transcription cofactor FgSwi6 plays a role in growth and development, carbendazim sensitivity, cellulose utilization, lithium tolerance,

deoxynivalenol production and virulence in the filamentous fungus *Fusarium graminearum*. *Fungal Genet. Biol.*, 58–59, 42–52;

MOGHTADER M., 2012 - Antifungal effects of the essential oil from *Thymus vulgaris* L. and comparison with synthetic thymol on *Aspergillus niger*, *Journal of Yeast and Fungal Research* Vol. 3(6), pp. 83 - 88, Available online at http://www.academicjournals.org/JYFR DOI: 10.5897/JYFR12.023 ISSN 2141-2413 ©2012 Academic Journals

MORCIA C., MALNATI M., TERZI V., 2011 - In vitro Antifungal Activity of Terpinen-4-ol, Eugenol, Carvone, 1,8-cineole (eucalyptol) and Thymol against Mycotoxigenic Plant Pathogens. *Food Addit. Contam*. A, 29, 415–422.

NGUEFACK J., LETH V., AMVAM ZOLLO P. H., MATHUR S. B., 2004 - Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. *Int J Food Microbiol 94*: 329-334.

RAHIMIFARD N., SH. SHOEIBI, SABZEVARI O., SOHEILA AJDARY, H. HAJIMEHDIPOOR, S. R. PAKZAD, M. PIRALI HAMEDANI, F. BAGHERI, B. ESMAEILI, 2008 - Antifungal activity of the native essential oil of *Thymus vulgaris* on *Candida albicans, Aspergillus niger* and *Aspergillus flavus* from Iran, *Journal of Pure and Applied Microbiology*, vol. 2 (2), p. 343 - 346;

RASOOLI I., REZAEI M. B., ALLAMEH A., 2006 - Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock. Food Control 17*: 359-364.

RAZZAGHI - ABYANEH M. M., SHAMS - GHAHFAROKHI M. B., REZAEE K., JAIMAND S., ALINEZHAD R., SABERI R., YOSHINARI T., 2009 - Chemical composition and antiaflatoxigenic activity of *Carum carvi* L., *Thymus vulgaris* and *Citrus aurantifolia* essential oils. *Food Control 20*: 1018-1024.

REDDY K. R. N., NURDIJATI S. B., SALLEH B., 2010a. - An overview on plant derived products on control of mycotoxigenic fungi and mycotoxins. *Asian J Plant Sci 9*: 126-133.

SILVA, FERNANDA C. DA, CHALFOUN, SARA M., SIQUEIRA, VIRGÍNIA M. DE, BOTELHO, DEILA M. DOS S., LIMA, NELSON, & BATISTA, LUÍS R., 2012 - Evaluation of antifungal activity of essential oils against potentially mycotoxigenic *Aspergillus flavus* and *Aspergillus parasiticus. Revista Brasileira de Farmacognosia*, *22*(5), 1002-1010. Epub APRIL 17, 2012.HTTPS://DX.DOI.ORG/10.1590/S0102-695X2012005000052;

SOLIMAN K. M., BADEA B. I., 2002 - Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food Chem Toxicol 40*: 1669-1675.

STARKEY D. E., WARD T. J., AOKI T., GALE L. R., KISTLER H. C., GEISER D. M., SUGA H., TOTH B., VARGA J., O'DONNELL K., 2007 - Global molecular surveillance reveals novel *Fusarium* head blight species and trichothecene toxin diversity. *Fungal Genet. Biol.* 2007, 44, 1191–1204.

SUN H. Y., ZHU Y. F., LIU Y. Y., DENG Y. Y., LI W., ZHANG A. X., CHEN H. G., 2014 - Evaluation of tebuconazole for the management of *Fusarium* head blight in China. *Australas. Plant Pathol.*, 43, 631–638.

ZHANG H., VAN DER LEE T., WAALWIJK C., CHEN W., XU J., ZHANG Y., FENG J., 2012 - Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. *PLoS ONE* 2012, 7, e31722.