Scientific Papers. Series D. Animal Science. Vol. LXIV, No. 2, 2021
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

FUNGI AND MYCOTOXINS CONTROL OF WHEAT GRAINS USING ESSENTIAL OILS

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Abstract

The paper aims to study the antifungal and antimycotoxigenic effect of some essential oils (garden thyme, oregano, coriander, dill and fennel) on wheat seeds. In order to test the protective effect associated with treatment with essential oils (EOs), wheat seeds naturally contaminated with deoxynivalenol (DON) were sprayed with different concentrations of EOs, and after 7 days and 14 days the seed contamination index (SCI), fungal genera and DON content were determined. The obtained results showed that the seed contamination index (SCI), after one week of treatment, is higher than control in case of fumigation of wheat seeds with oregano and fennel essential oil and lower than control in case of coriander, thyme and dill essential oils. The predominant fungal species in this phase are: Fusarium, Cladosporium and Rhizopus. Two weeks after treatment, it is observed that the treatment with essential oils provides fungal protection. SCI is maximum in the case of control and the potential to inhibit micellar colonization increases in the order: fennel <dill = oregano <coriander <thyme. With the exception of dill oil, which did not reduce Fusarium contamination, the other essential oils provide a significant reduction in the number of seeds contaminated with this type of fungus. Regarding the antimycotoxigenic effect, the level of DON decrease after treatment with essential oils, in all experimental variants tested, the decrease being more pronounced after 14 days after treatment, compared to 7 days.

Key words*: Coriander, Deoxynivalenol, Dill, Fennel, Oregano, Thyme.*

INTRODUCTION

Infection of cereal grains by fungi is a serious problem worldwide, phenomenons that reduces yield, quality and nutritional value of cereals and develop the production of mycotoxins that are harmful to both humans and animals (Petcu et al., 2019). Preventing contamination with pathogens through the use of essential oils is considered a viable non-polluting strategy to reduce the risks associated with mycotoxin contamination of processed food and feed.

Previous studies draw attention to the functionality of essential oils (EOs) of medicinal plants in term of fungal contamination of cereals studied and proven by researches in this regard (Naeini et al., 2010; Isman et al., 2000; Quiroga et al., 2001; Sumalan et al., 2013). The inhibition potential of some EOs against natural mycoflora and *Fusarium* mycotoxins production has been associated with the chemical composition, monoterpenic phenols, especially thymol, carvacrol and eugenol in the oils (Soliman et al, 2002; Lee et al., 2007).

The EOs of *Thymus vulgaris* (TEO) and *Coriandrum sativum* (CEO) inhibited the growth both for filamentous fungi and yeasts that are involved in the postharvest spoilage of wheat seeds. After 5 days of treatment CEO, SCI were maximum (33.33%) for a level of 500 ppm and decreases (30%) for a level of 1000 ppm, respectively at 20% for a level of 2000 ppm (Sumalan et al., 2009).

Regarding the *Thymus vulgaris* oil (TEO) the SCI values were in the range 36.67-20% depending on the applied level (Alexa et al., 2018). Similar results on the antifungal effect and complete inhibition of the growth of *Aspergillus flavus, Fusarium osysporum, Curvularia lunata, Aspergillus terreus, Aspergillus fumigantus, Alternaria and*

Cladosporium sp. of TEO have been reported previously (Dambolena et al.; 2008, Kumar et al., 2008).
Other in vitro study (Negrea et al., 2018a) highlighted the fungistatic and fungicidal effect of oregano essential oil (OEO) against *Fusarium graminearum* at lower concentrations: minimum concentration with fungistatic effect - 0.06%, minimum concentration whit fungicidal effect - 0.1% and fungicidal concentration - 0.2%.
High volatility, absence of toxicity and especially their antimicrobial effects recommend the use of natural preparations based on EOs in organic agriculture and horticulture.
The aim of this study is to analyze the antifungal and antimycotoxignic effect of different essential oils: garden thyme (TEO), oregano (OEO), coriander (CEO), dill (DEO) and fennel (FEO) on wheat seeds in order to use these natural compounds as protective agents in grain warehouses. Also, the effect of treatment on wheat germination was tested.

MATERIALS AND METHODS

Sample preparation

For the experiment Antille variety wheat obtained by ecological technology was used. In order to test the protective effect associated with the treatment with EOs, 50 g of wheat sample (with a known DON concentration-0.464 ppm) were sprayed with different concentrations of EOs and after 7 days and 14 days were analyzed to detect the seed contamination and DON content.
The moisture content of the sample was 12.41% and the water activity index (a_w) was 0.9.
The oils used were: garden thyme (TEO) - 0.03%; oregano (OEO) - 0.03%; coriander (CEO) - 0.2%; dill (DEO) - 0.2%; fennel (FEO) - 0.2%. The concentrations were selected after the in vitro test regarding minimum inhibitory concentration (MIC) of each EOs (Negrea et al., 2018a; Negrea et al., 2018b).

Determination of seed contamination index (SCI) and identification of fungal genera

The observations were made after 7 and 14 days by estimating the degree of seed germination and the seed contamination index (SCI) for each treatment variant following the procedure of Doolotkeldieva et al. (2010).

$$\text{SCI}\ (\%) = \frac{\text{contaminated seeds}}{\text{total seeds}} \cdot 100$$

Identification of fungal genera

The identification of fungus genera has been performed according to Hocking et al. (2006).
The frequency of occurrence of the fungal genera (Fr) was calculated using formula (Doolotkeldieva, 2010):

$$\text{Fr}\ (\%) = \frac{\text{number of seeds with fungal genus}}{\text{total number of seeds}} \cdot 100$$

Determination of mycotoxins:

The method used was the enzyme-linked immunosorbent assay (ELISA), and sample preparation was performed in accordance with the manufacturer's instructions for analysis of deoxynivalenol (DON) in cereals (R-Biopharm). The ground sample (5 g) was extracted with 100 ml of distilled water and homogenized using a stirrer 20 min for extraction. The extract was filtered, and 1 ml of the filtrate was used directly for enzymatic analysis. Standard solutions and samples (50 µl) are mixed with 50 µl enzyme conjugate in individual cells from the ELISA kit plates. 50 µl of antibody solution are added and incubated for 10 minutes at room temperature. The cells were washed three times with 250 µl of distilled water, then the substrate (100 µl) was added and incubated for another 5 minutes at room temperature. The stop solution (100 µl) was added to each cell and the yellow color intensity was measured at 450 nm using an ELISA 96 reader (PR-1100, Bio-Rad Laboratories, USA).

Determination of wheat germination

In order to study the effect of treatment with EOs against wheat germination, wheat samples were sterilized by using sodium hypochlorite solution (1:9), and washed two times with distilled water. A total of 10 seeds were placed in a Petri dish. Then, the seeds were treated with each EOs in the same concentrations used for antifungal experiment. The Petri dishes were kept at 25◦C into the dark. After seven days, the number of germinated wheat seeds was recorded. A seed was considered

germinated when the radicles was elongated up to 3 mm (Alexa et al., 2018). The number of germinated seeds was divided to the number of seeds used (10) and that multiplied with 100, resulting the percentage of germinated seeds (GS).

RESULTS AND DISCUSSIONS

Seed contamination index (SCI) and identification of fungal genera

Fungal seed colonization index (SCI) after 1 week and after 2 weeks of fumigation with essential oils (EOs) is presented in Figure 1.

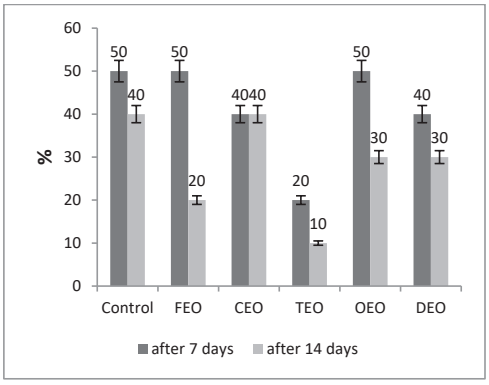

Figure 1. SCI (%) after 7 days and 14 days after fumigation with EOs

Figure 1 shows that SCI after one week of treatment is as the control in case of fumigation of wheat seeds with OEO and FEO and lower than the control in case of use of CEO, DEO and TEO. The lowest SCI (20%) was recorded in the case of TEO treatment. The inhibition potential of micellar colonization increases in the order: FEO < DEO = OEO < CEO < TEO. After 14 days, the SCI decrease for all samples, excepting CEO. The SCI was 30% when DEO and OEO was applied, 20% for FEO and 10% for TEO. The results highlighted the maximal antifungal potential of TEO after 14 days of treatment.

The inhibition capacity of TEO was proven even after 5 days of treatment when the growth of molds in wheat samples was inhibited in the range of 36.67-20% depending on the applied level (Sumalan et al., 2013). Similar results on the antifungal effect of TEO have been reported by Dambolena et al. (2008) and Kumar et al. (2008).

The inhibition rate depends on applied concentration of EOs. In the case of treatment with CEO, after 5 days, SCI values were 33.33% for a level of 500 ppm, 30% for a level of 1000 ppm and 20% for a level of 2000 ppm (Sumalan et al., 2013).

The predominant fungal species after 7 days of treatment with EOs are: *Fusarium, Cladosporium and Rhizopus* (Figure 2). Two weeks after the treatment, it was observed that the treatment with EOs provides fungal protection. At this stage, the predominant fungal species is *Alternaria* and fungal strains *Aspergillius, Penicillium and Fusarium* are also found in control. With the exception of DEO, which did not reduce *Fusarium* contamination, the other analyzed EOs provides a significant reduction in the number of seeds contaminated with this type of fungus.

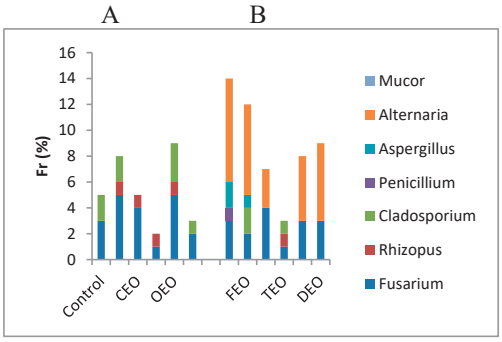

Figure 2. The frequency of occurrence of the fungal genera (Fr) after 1 week (A) and after 2 weeks (B) of fumigation with essential oils (EOs)

TEO was proven its maximal antifungal potential after 14 days of treatment, followed by CEO, OEO, DEO and FEO.

The antifungal potential of TEO has been previously demonstrated by our research team against *Fusarium graminaerum* (Alexa et al., 2018). Also, the antifungal effect of TEO against *Verticillium dahliae, Fusarium* sp., *Penicillium* sp., *and Aspergillius* sp. was reported (Arslan et al., 2010; Kocic-Tanackov et al., 2013).

The results are in accord with previously data reported in literature. In this regard, other study highlighted that the treatment with CEO doesn't inhibit the occurrence of fungi such as *Alternaria, Fusarium, Aspergillus and Hyphopichia*. Only at high concentration

(2000 ppm) it was noticed to decrease the fungus frequency (Sumalan et al., 2013). Opposite, TEO exhibited a broad spectrum fungitoxicity against: *Fusarium, Cladosporium and Aspergillus* (Sumalan et al., 2013), *Aspergillus flavus, Fusarium osysporum, Curvularia lunata, Aspergillus terreus, Aspergillus fumigantus, Alternaria and Cladosporium s*p. (Kumar et al., 2008) and *Aspergillus niger, A. ochraceus and A. flavus* (Bluma et al., 2008) by treatment with different concentrations of TEO.

DON inhibition

Figure 3 shows the level of DON contamination after 7 and 14 days of treatment. It can be noted that the DON concentration is lower as in Control for all analyzed samples. The initial level of DON was 0.464 ppm and decreased in control, after 7 days at 0.282 ppm, while in the wheat samples with EOs treatment the concentration of DON is lower (between 0.141-0.199 ppm). The higher antimicotoxigenic potential was proven by DEO, followed by FEO, TEO, CEO and OEO. After 14 days of treatment the inhibition effect of EOs against DON increased. The profile of DON was same as after 7 days of treatment and highlights the maximal antimicotoxigenic potential of DEO (0.027 ppm) and de minimal effect when OEO was applied (0.118 ppm). The results shown that the EO with the highest antifungal effect is not the one with the highest antimicotoxigenic effect. TEO was proven to inhibit the fungal activity but had a moderate action in terms of DON inhibition.

Responsible for the antifungal effect of EOs can be the changes induced on the fungal morphogenesis and fungus growth produced by the chemical compounds of EOs (Rassoli et al., 2006). The effect of major chemical compounds on antifungal activity varies as follow: phenols > alcohols > aldehydes > ketones > ethers > hydrocarbons (Singh et al., 2012).

Figure 3 shows that the level of DON contamination decreases after treatment with EOs, in all experimental variants tested, the decrease being more pronounced after 14 days of treatment, compared to 7 days. The maximum efficiency is observed in the case of treatment with EOs from the *Umbelifaere* family (DEO> FEO> CEO), followed by oils from the *Lamiaceae* family (TEO> OEO). The reduction of DON contamination of wheat seeds is 29.43-50.52% after 7 days of treatment, respectively between 55.47-89.81% after 14 days of treatment.

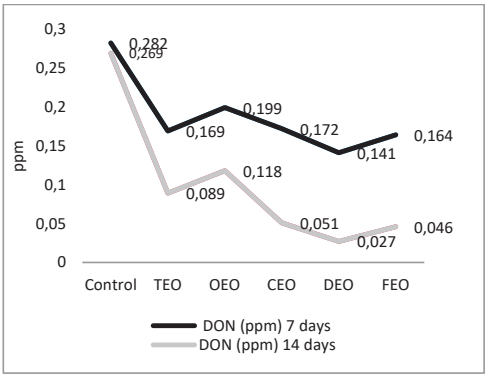

Figure 3. DON content (ppm) after 7 days and 14 days of treatment with EOs

Seeds germination

The effect of EOs on seed germination is presented in the Figure 4. It can be seen 10% non-geminated seeds in the case of treatment with CEO, OEO or FEO and 20% when DEO was applied on wheat control.

The effect of TEO on wheat germination reported in previous study (Alexa et al., 2018), highlighted that 0.3-1% TEO inhibited 40% of seed germination. The use of lower concentration of TEO (0.03%), concentration that was proved with antifungal effect, don`t affect the wheat germination. This aspect is useful in choosing the concentrations used in the field in the protection of agricultural crops, so that the antifungal effect is maximum and the germination of seeds is not affected. Regarding other EOs analyzed, the tested concentrations with antifungal effect, leads to a germination seed coefficient (GS) over 80%, without significant effect on wheat germination.

Previous study regarding the effect of FEO on wheat seeds germination shows that the rate of inhibition varies between 88.88-61.11%, depending on the concentration level (1-0.3%) (Negrea et al., 2018b). The lower concentration used in our study (0.2%), that proven a fungicidal effect, inhibited just 10% of seed in term of germination.

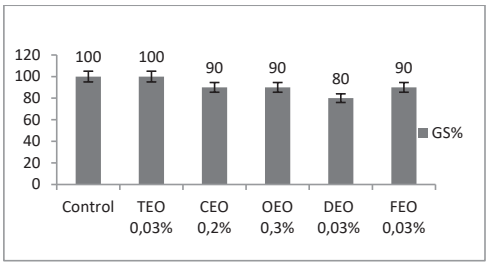

Figure 4. The effect of EOs treatment on seeds germination

CONCLUSIONS

Our results highlight the antifungal potential of EOs, especially those of the *Lamiaceae* family (garden thyme and oregano), and the antifungal effects against DON exhibited by EOs from *Umbeliferae* family (dill and coriander).
Wheat germination capacity is not affected by treatment with EOs, that recommends them as potential natural antifungal and antimycotoxigenic agents with applicability in agriculture and protection of cereals and plant raw materials used in the food industry.

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