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Effect of fungicidal treatment and storage condition on content of selected mycotoxins in barley
Vliv fungicidního ošetření a podmínek skladování na hladiny vybraných mykotoxinů u ječmene

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The aim of the study was to determine the effect of fungicidal treatment and storage on the occurrence of mycotoxins in barley (*Hordeum vulgare L.*). Barley was initially inoculated with *Fusarium culmorum* followed by the application of fungicides (prothioconazole and bixafen). A screening of 57 mycotoxins were performed using ultra-performance liquid chromatography in tandem with mass spectrometry. The fungicide treatment affected (P <0.05) the levels of zearalenone, β-zearalenol, arternariol and alternariol-methylether that were present. Levels of deoxynivalenol was highest in the second year of monitoring. 3-acetyl-deoxynivalenol was not affected by fungicidal treatment or storage. The significant increase (P <0.05) of DON-3-glucoside, 15-acetyl-DON, enniatin A, enniatin A1, enniatin B, and enniatin B1 was measured in barley samples. The results of the experiment determined that the use of fungicides can suppress some kinds of mycotoxins, but not others.

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Cílem studie bylo zjistit vliv fungicidního ošetření a skladování na výskyty mykotoxinů u ječmene (*Hordeum vulgare L.*). Ječmen byl v prvním kroku inokulován *Fusarium culmorum*, následně byly aplikovány fungicidy (prothiokonazol a bixafen). Celkově bylo sledováno 57 mykotoxinů s použitím vysokoúčinné kapalinové chromatografie v tandemu s hmotnostní spektrometrií. Fungicidní ošetření ovlivnilo hladinu zearalenonu, beta-zearalenolu (P <0,05), arternariolu a alternariolu-methyletheru. Hladiny deoxynivalenolu byly vyšší ve druhém roce skladování. Obsah 3-acetyl-deoxynivalenolu nebyl ošetřením ani skladováním ovlivněn. Dále bylo sledováno ve vzorcích ječmene zvýšení (P <0,05) DON-3-glukosidu, 15-acetyl-DON, enniatinu A, enniatinu A1, enniatinu B a enniatinu B1. Výsledky experimentu ukázaly, že použití fungicidů může potlačit některé druhy mykotoxinů, není ovšem účinné na všechny mykotoxiny.

Keywords: *bixafen, enniatin, Fusarium culmorum, Hordeum vulgare, deoxynivalenol, prothioconazole*
Klíčová slova: *bixafen, enniatin, Fusarium culmorum, Hordeum vulgare, deoxynivalenol, prothiokonazol*

1 INTRODUCTION

Mycotoxins are secondary metabolites that are produced by micromycetes that usually parasitically or saprophytically live on agricultural crop products (Calado et al., 2014). Mycotoxin contamination may not be noticeable, but they contribute to chronic, acute intoxication, or even death of animals and humans if they are consumed. Acute toxicosis is caused by the intake of high doses and most often causes degeneration of the liver, kidneys, damage to the digestive tract, the circulatory system and the central nervous system. Chronic exposure is caused by long-term low dose intake. The impact could be teratogenic, mutagenic and carcinogenic effects on the organism, weakening of the immune system and reduction of animal performance (Degen, 2017). Mycotoxins posing the highest risk as feed and food contaminants belong to the groups of aflatoxins, trichothecenes, fumonisins, zearalenones, ochratoxins and ergot alkaloids. In addition, synergistic toxic effects caused by a combination of several different mycotoxins have been demonstrated (Battilani, 2016; Kamala et al., 2017; Oswald et al., 2005; Vejdovszky et al., 2017).

Barley is a major cereal grain used as animal fodder, as a source of fermentable material for beer and certain distilled beverages, and as a component of various health foods. *Fusarium culmorum* and *Fusarium gravinearum* are the most common pathogenic mold that can contaminate grain crops. In the case of beer production, *Fusarium* spp. causing over foaming of beer (referred to as gushing) (Mastanjević et al., 2018). Moreover, cereal disease such as Fusarium head blight is responsible for the reduction of grain yields worldwide (Wolf-Hall, 2007). Contaminated grains has a decreased ability of malting with the simultaneous deterioration of other quality parameters such as Kolbach index, protein content, β-glucan content, malt extract, extract difference, saccharification time, wort colour, and viscosity (Pascari er al., 2018). In addition, there is an increased heath risk due to the production of mycotoxins that are exceptionally durable against heat and chemical treatments.

A number of strategies for the reduction and control of mycotoxins have been considered in different areas of world (Bartos et al., 2017; Battilani et al., 2014). The control of mycotoxins involves prevention of mold growth in crops and other feedstuffs and the use of decontamination of mycotoxin contaminated feeds/foods as a secondary strategy (Magan and Aldred, 2007; Reverberi et al., 2010; Skladanka et al., 2011) Fungicides such as prothioconazole, spiroxamine, tebuconazole or bixafen are commonly used to eliminate mold and mycotoxins in barley production (Skladanka et al., 2011).

The aim of this study was to evaluate the effect of fungicidal treatments and storage on the occurrence of mycotoxins in barley during storage. The presence of 57 mycotoxins, in barley samples using ultra-performance liquid chromatography with mass detection was examined.

2 MATERIALS AND METHODS

2.1 Chemicals

Methanol, ammonium acetate, formic acid, acetonitrile, magnesium sulphate and mycotoxins standards (Fusarenon X, nivalenol, deoxynivalenol, alfa-zearalenol, beta-zearalenol, zearalenon, 3-acetyl-

deoxynivalenol, patulin, alternariol, alternariol-methylether, deoxynivalenol-3-glucoside, enniatin B, enniatin B1, enniatin A, enniatin A1, ergokornin, ergokorninin, ergokristin, ergokristinin, ergokryptin, ergokryptinin, ergosin, ergosinin, ergometrin, ergotamin, ergotaminin, agroklavin, neosolaniol, diacetoxyscirpenol, fumonisin B1, fumonisin B2, fumonisin B3, 15-acetyl-deoxynivalenol, aflatoxin B1, aflatoxin B2, aflatoxin G2, aflatoxin G1, HT-2 toxin, T-2 toxin, sterigmatocystin, ochratoxin A, citrinin, beauvericin, cyclopiazonic acid, mycophenolic acid, penicillic acid, rockfortin C, tentoxin, tenuazonic acid, verrucarol, verruculogen, penitrem A, stachybotrylaktam, phomopsin A, gliotoxin, meleagrin, paxillin) were purchased from Sigma-Aldrich (St. Louis, USA) in ACS purity unless otherwise noted. Antifungal treatment (*Table 1*) were purchased from Bayer (Leverkusen, Germany).

Table 1 Composition of antifungal treatment

Hutton		Prosaro 250 EC		Zantara	
$g.L^{-1}$		$g.L^{-1}$		$g.L^{-1}$	
100	prothioconazole	125	prothioconazole	50	bixafen
250	spiroxamin	125	tebuconazole	166	tebuconazole
100	tebuconazole				

2.2 Barley cultivation

Barley (Sebastian variety) samples (Libcany area, Czech Republic – European Union) were artificially treated with *Fusarium culmorum* (WGSm. Sacc. Strain KM16902; DON chemotype). The inoculation was carried out with a conidia suspension of the pathogenic isolate of *F. culmorum* (concentration of 0.5 mil. conidia/1 mL of inoculum; spray dose of 200 L/ha). The inoculation was performed at the optimal vegetative phase according to the methodology by Tvarůžek et al. (2012). The treated group of barley (A variant) was treated with Hutton (0.8 l/ha at BBCH of 36) + Zantara (1.5 l/ha, BBCH of 65). Another group (B variant) was treated with the combination of Hutton (0.8 l/ha, BBCH of 36) + Prosaro EC250 (0.75 L/ha, BBCH of 65). The control group, used for comparison with the treated samples, was untreated. The control group was grown on the same plot and was in the same developmental phase as the treated groups.

2.3 Standards preparation

Solid standards were dissolved in acetonitrile or methanol prior to use and stored together with liquid standards at –18 °C. From stock solutions were prepared working solutions with the concentration 1000 ng/mL. Individual calibration solutions (0.1 to 1000 ng/mL) were prepared by removing the calculated volumes from the working standard containing all mycotoxins in the vials, blowing acetonitrile with a gentle stream of nitrogen and dissolving in 1 ml of blank extract of the sample. Calibration solutions of the matrix standards were stored in the freezer at -18 °C.

2.4 Samples storage

Barley was harvested at full maturity. Barley samples were stored under the following defined conditions: relative humidity 70 ± 1%, temperature 18 ± 1 ° C (the temperature was maintained by active ventilation), dark (0 lux), grain humidity 14 ± 0.5%. Barley was stored freely with a sample size of 15 kg.

2.5 Samples preparation

A total of 2 g of barley was weighed to PTFE centrifuge tubes (50 ml) followed by the addition of 10 ml of acidified distilled water (0.2% formic acid). The mycotoxin extraction was performed by placing the samples for 30 min on a magnetic stirrer (10000 rpm) with the addition of 10 ml of acetonitrile. Subsequently, 4 g $MgSO_4$ and 1 g NaCl were added to the mixture and vigorously shaken by hand. The samples were centrifugated (5 min, 10000 g) and the supernatants were purified using microfilter (0.2 μm porosity).

2.6 Ultra-performance liquid chromatography with MS detection

For the identification and quantitative determination of the mycotoxins, Acquity UPLC® System (Waters, Milford, MS, USA) in connection with a tandem mass spectrometer QTRAP® (AB Sciex, Ontario, Canada). The program Analyst ® (Thermo Fisher Scientific) was used for data processing. Separation was performed using Acquity UPLC® HSS T3 reverse phase column (100 x 2.1 mm, 1.8 µm; Waters). For mycotoxins analysis were used protocol according to Sumíková et al. (Sumikova et al., 2017). The separation conditions were as follows: temperature 40 °C with a sample injection volume of 2 µl. Mycotoxins were separated by gradient elution. Mobile phase A consisted of 5 mM ammonium acetate and mobile phase B was 100% methanol. The detection was carried out using QTRAP 5500 (AB Sciex, Ontario, Canada). The conditions of ionization were in MRM detection mode 60 s. The voltage on the electrospray was -4500V/+4500V and the temperature was at 500 °C/600 °C.

2.7 Statistics

The data were statistically analysed using STATISTICA.CZ, version 10.0 (the Czech Republic). The results were expressed as mean ± standard deviation (SD). Statistical significance was determined using ANOVA and Scheffé's test (one-way analysis). The differences with $P<0.05$ were considered to be significant.

3 RESULTS

The barley samples were analysed using ultra-performance liquid chromatography in tandem with mass spectrometry detection. The method has been optimized for 57 kinds of mycotoxins based on our previous research. (Horky and Cerkal, 2014) (Sumikova, et al., 2017).

3.1 Determination of the mycotoxins presence in the barley samples

Barley samples were screened for 57 mycotoxins of microscopic filamentous fungi of the genus *Fusarium*, *Penicillium, Aleternaria* and *Aspergillus*. In addition, a focus of the study was on the the analysis of masked mycotoxins. A total of 12 mycotoxins were detected of which 10 are mainly produced by *Fusarium* spp. : deoxynivalenol (DON), 3-acetyl-DON, DON-3-glucoside, 15-acetyl-DON, zearalenone, β-zearalenol, enniatin B, enniatin B1, enniatin A and enniatin A1). However, other mycotoxins produced by *Fusarium* spp., such as beauvericin, diacetoxyscirpenol, fumonisin -B1 -B2 -B3, HT-2 toxin, neosolaniol, nivalenol, T -2 toxin and fusarenon X, were not detected. The presence of alternariol and alternariol-methylether indicates the presence of *Alternaria* spp. A total of 50% of all detected mycotoxins is DON in the control sample (*Fig. 1*). The presence of the following mycotoxins in the samples was not found: tentoxin, tenuazonic acid (*Alternaria* spp.), aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, gliotoxin, ochratoxin A, patulin, sterigmatocystin (*Aspergillus* spp.), agroklavin, ergokornin, ergokorninin, ergokristin, ergokristinin, ergokryptin, ergokryptinin, ergometrin, ergosin, ergosinin, ergotamine, ergotaminin (*Claviceps* spp.), phomopsin A (Diaporthe spp.), verrucarol, verruculogen (*Myrothecium* spp.), citrinin, meleagrin, mycophenolic acid, paxilline, penicillin acid, penitrem A, rokfortin, Cyclopiazonic acid (*Penicillium* spp.) and stachybotrylaktam (*Stachybotrys* spp.)

3.2 The influence of antifungal treatment on *F. culmorum* mycotoxin production

Statistical analysis was used to evaluate the mycotoxin content in the barley samples. The infected plants were treated by two antifungal agents; A Hutton (prothioconazole 100 g/L) with Prosaro 250 EC (prothioconazole 125 g/L) and B Hutton with Zantara (bixafen 50 g/L). Figure 2 compares the results obtained from the analysis of A) DON, B) DON-3-glucoside, C) 3-acetyl-DON, D) 15-acetyl-DON, E) ZEA and F) β-ZEA after harvest (year 2016) and 1 storage (year 2017). There was strong evidence of effectiveness of different antifungals and storage. Deoxynivalenol was increased by 2023 µg/kg ($P<0.05$) in A variant after fungicidal treatment. During storage, significant increase, up to 19850 µg/kg of DON, was observed in the case of treatment B (*Fig. 2A*;$P<0.05$). The level of deoxynivalenol-3-glucoside was not affected by the fungicidal treatment. For all groups, a significant increase was detected during storage. The increase was approximately 2129 µg/kg ($P<0.05$) in a control group and up to 4103 µg/kg ($P<0.05$) in treatment A and 1535 µg/kg ($P<0.05$) in treatment B (*Fig. 2B*). 3-acetyl-deoxynivalenol, was not significantly affected by fungicidal treatment or storage duration (*Fig. 2C*). 15-acetyl-deoxynivalenol increased by 29 µg/kg ($P<0.05$) in treatment A after the application of fungicides. During storage, a decrease of 15-acetyl-deoxynivalenol of 25 µg/kg ($P<0.05$) in the control group, compare to approximate 46 µg/kg ($P<0.05$) in treatment A and up to 18 µg/kg ($P<0.05$) in treatment B was observed (*Fig. 2D*). Zearalenone was reduced by 1331 µg/kg ($P<0.05$) after the use of fungicides in treatment B. During storage, zearalenone in the control group and treatment B increased by 1540 µg/kg ($P<0.05$) and

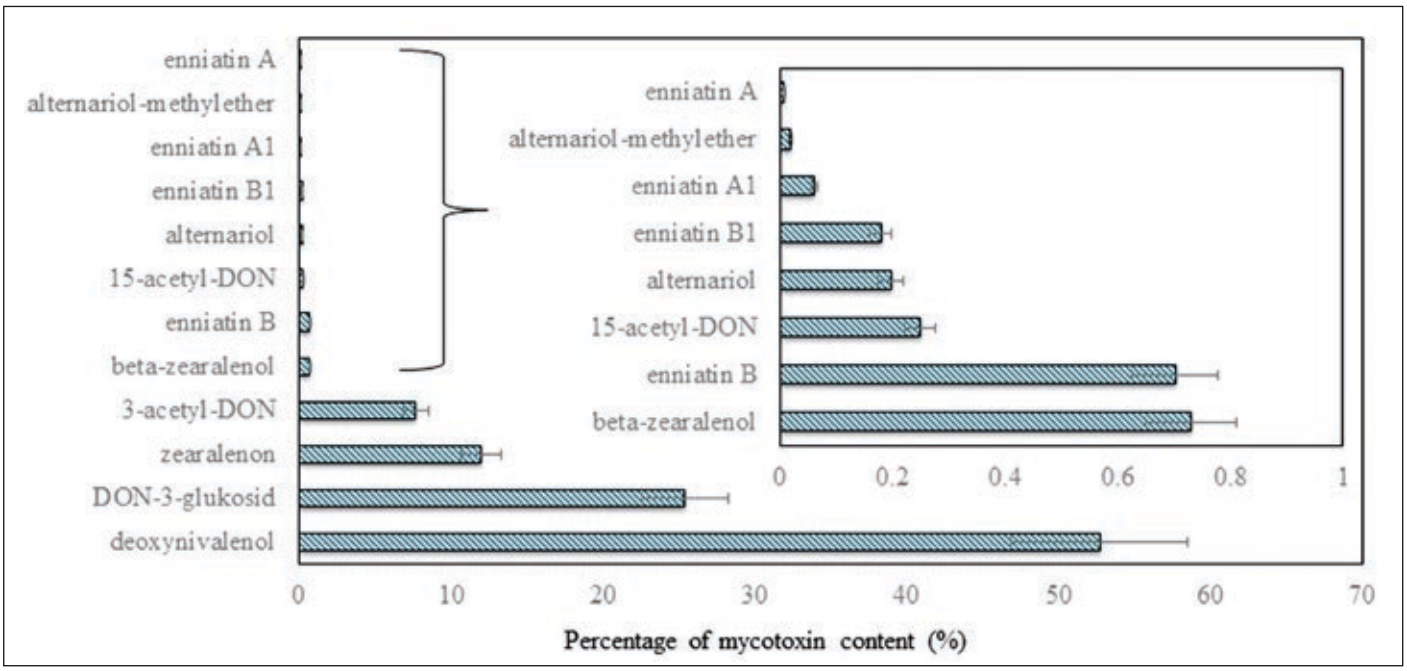

Fig. 1 Comparison of the analysed mycotoxins content with the sum of all occurrences of mycotoxins equaling 100%.

2081 µg/kg (P <0.05), respectively (*Fig. 2E*). The level of β-zearalenol mycotoxin was reduced by 64 µg/kg (P <0.05) after the use of fungicides in treatment B. During storage, a significant decrease by 70 µg/kg (P <0.05) of β-zearalenol was detected in treatment A (*Fig. 2F*).

3.3 The influence of antifungal treatment on *F. culmorum* minor mycotoxins

Mycotoxins, such as enniatin B, significantly increased by 227 µg/kg in treatment A (P <0.05) and by 350 µg/kg (P <0.05) in treatment B after the application of fungicides. After storage, the enniatin B level increased by 263 µg/kg (P <0.05) in the control group (*Fig. 3A*). Enniatin B1increased in treatment A by 60 µg/kg (P <0.05) and by 109 µg/kg (P<0.05) treatment B following the application of fungicides. During storage, enniatin B1 increased by 81 µg/kg (P <0.05) in the control group (*Fig. 3B*). The enniatin A1 level increased in treatment A after the treatment with fungicide by 21 µg/kg (P <0.05) and by 36 µg/kg (P<0.05) treatment B. During storage, a significant increase of enniatin A1 by 15 µg/kg (P <0.05) was detected in the control group (*Fig. 3C*). Enniatin A was significantly increased by 3

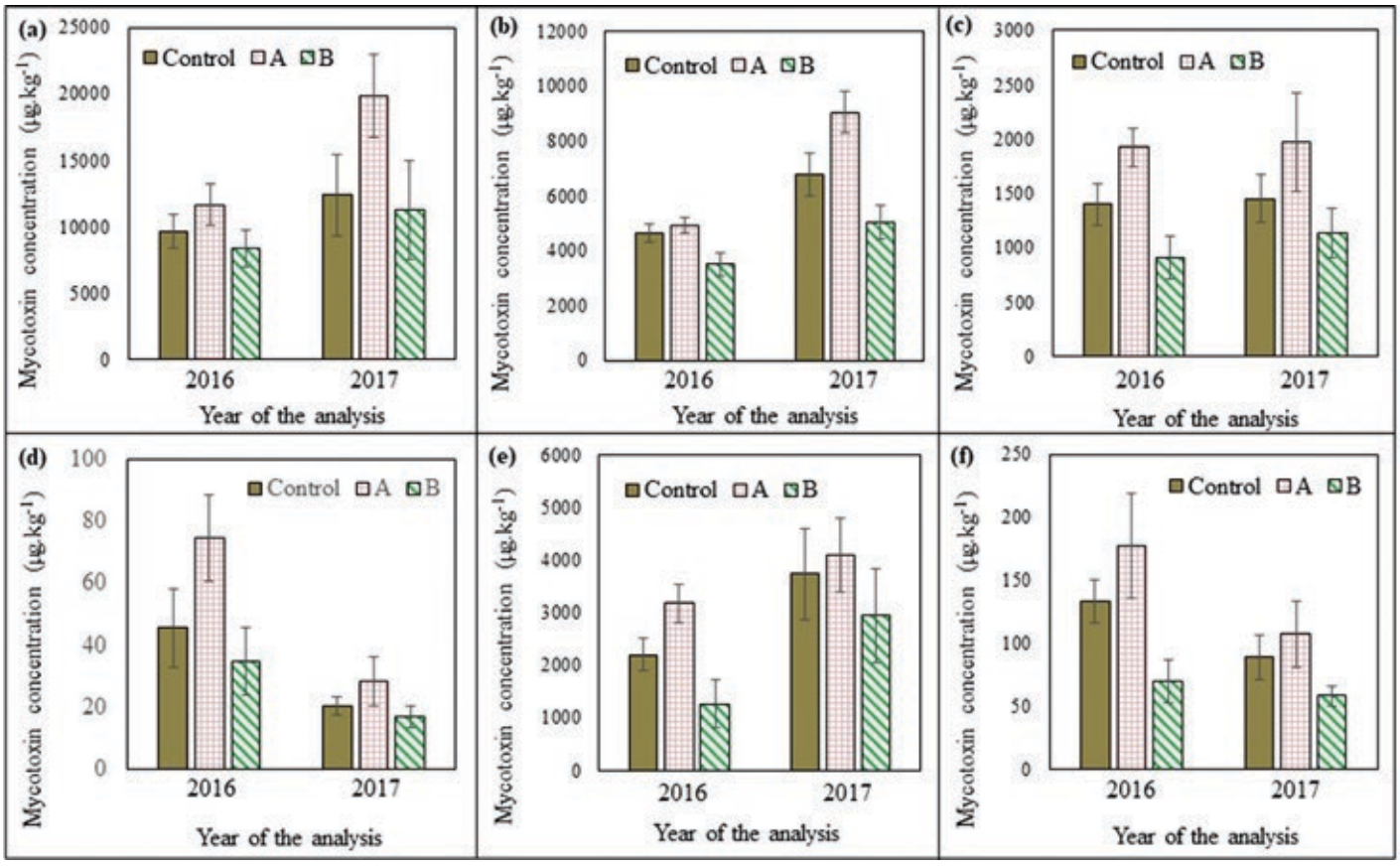

Fig. 2 Mycotoxin concentration following antifungal treatment. **A**) DON, **B**) DON-3-glucoside, **C**) 3-acetyl-DON, **D**) 15-acetyl-DON, **E**) ZEA, **F**) β-ZEA. Control group are barley samples without antifungal treatment application. Treatment A is Hutton (prothioconazole 100 g/L) with Prosaro 250 EC (prothioconazole 125 g/L) and Treatment B is Hutton with Zantara (bixafen 50 g/L)

µg/kg (P <0.05) after the application of fungicides in treatment A and by 5 µg/kg (P <0.05) in treatment B. During storage, enniatin A was increased by 4 µg/kg (P <0.05) in the control group (*Fig. 3D*). The level of alternariol mycotoxin reduced after the application of fungicides in Treatment Aa by 26 µg/kg (P <0.05) and by 24 µg/kg (P <0.05) in treatment B. The storage time did not significantly effect the alternariol content in barley (*Fig. 3E*). Similarly, the alternariol methyl ether content was reduced by 1.9 µg/kg (P <0.05) in treatment A and by 2 µg/kg (P <0.05) in treatment B following the application of fungicides. During storage, no significant changes occurred in the alternariol-methyl ether content of the individual barley samples (*Fig. 3F*).

4 DISCUSSION

Due to the artificial inoculation of *Fusarium culmorum*, the DON level was found to be high (8195 µg.kg^{-1} in comparison with control sample) and the fungicidal treatment proved to have no effect. Several different variants of fungicides can be used. According to the results obtained, all of the fungicides tested successfully suppressed fungal growth and mycotoxin production (Schmidt-Heydt et al., 2013). Based on these results, fungicides lose their functionality when an increased infestation of fungal diseases is present and the standard and masked mycotoxins in cereals can be increased (Popiel et al., 2017). Theinoculation of wheat by *Fusarium graminearum* and the use of fungicides decreased the DON by 86.5 % compared with the control group (Sip et al., 2010). According to Nakajima et. al. (Nakajima et al., 2008), it is appropriate to apply fungicides to the barley at a later grow stage. In this study, fungicides were applied in the 65 phase of phenological development stage, but we cannot confirm that this phase was optimal. Stanciu et al. (Stanciu et al., 2017) stated that the occurrence of enniatin B and enniatin B1 in cereals can be influenced by agro-technology. In Romania (the European Union), the values of enniatin B and enniatin B1 were detected from 170 to 815 µg/kg in the experiment. In this study, the values of these two mycotoxins ranged from 33 to 478 µg/kg. Paradoxically, the highest values were measured for fungicide-treated variants in the Enniatin group. However, the limit values have not yet been established for enniatin. In another study, a longer storage period of 20-24 weeks was required for the production of DON mycotoxin. Grain humidity is also important during storage with 12 to 14 % humidity optimal storage conditions (Atalla et al., 2003). High levels of mycotoxins (especially DON and ZEN) were measured in this study, even if the barley had an optimal humidity of 14%. In the case of strong fungal infestation (*Fusarium culmorum* was used with artificial inoculation in our experiment), fungicidal treatment and optimum grain humidity were not helpful. In another work, wheat was inoculated with *Fusarium culmorum* and then stored for 36 weeks. The DON level was not affected during storage. Nivalenol (NIV) was not found in any of the samples before storage, but appeared at the end of the experiment (Homdork et al., 2000). In this study, the level of DON was not significantly increased during storage and NIV mycotoxin was not identified in barley. Our study analysed a wide range of mycotoxins and lesser known mycotoxins (eg. enniatins), which are currently at the beginning of the research of their toxicity**.**

5 CONCLUSIONS

In this study, following mycotoxins were detected: deoxynivalenol, deoxynivalenol-3-glucoside, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, zearalenol, β-zearalenol, enniatin A, enniatin A1, enniatin B, enniatin B1, alternariol, alternariol, methyl-ether. Whereas levels of deoxynivalenol, zearalenone are monitored due to the legislative requirements, enniantins are not covered by the directives and regulations. The results from this study suggests there are likely the presence of mycotoxins following antifungal treatment indicating a need for the required detection of less known mycotoxins in food products.

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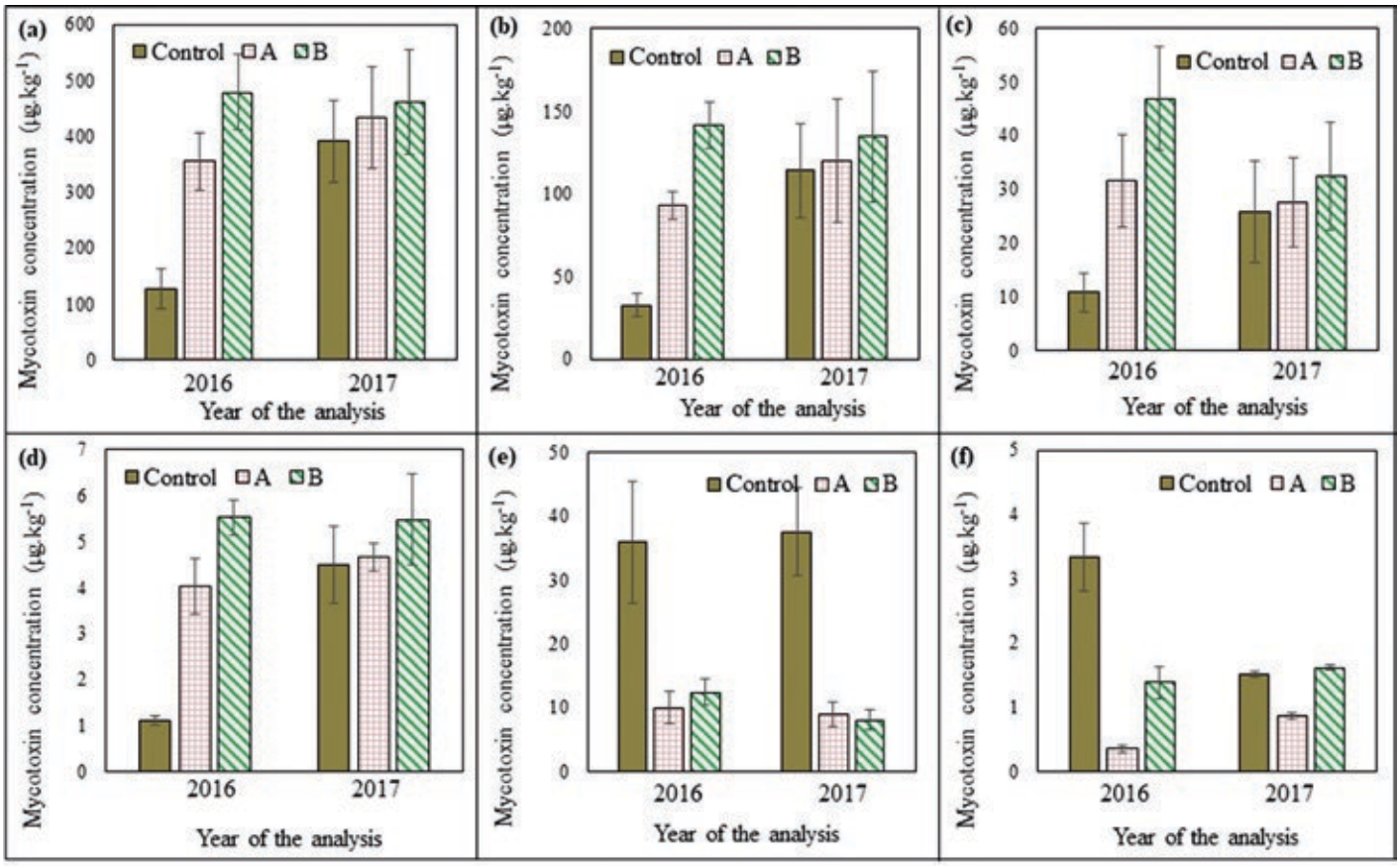

Fig. 3 Mycotoxin concentration following antigungal treatment. **A**) Enniatin B, **B**) Enniatin B1, **C**) Enniatin A1, **D**) Enniatin A, **E**) Altermariol, **F**) Alternariol – methylester. Control group are barley samples without antifungal treatment application. Treatment A is Hutton (prothioconazole 100 g/L) with Prosaro 250 EC (prothioconazole 125 g/L) and Treatment B is Hutton with Zantara (bixafen 50 g/L).

REFERENCES

Atalla, M. M., Hassanein, N. M., El-Beih, A. A., Youssef, Y. A. G., 2003: Mycotoxin production in wheat grains by different Aspergilli in relation to different relative humidities and storage periods. Nahrung-Food, 47: 6–10.

Bartos, P., Kriz, P., Havelka, Z., Bohata, A., Olsan, P., Spatenka, P., Curn, V., Dienstbier, M., 2017: Plasma technology in food industry: mini-review. Kvasny Prum., 63: 134–138.

Battilani, P., 2016: Recent advances in modeling the risk of mycotoxin contamination in crops. Current Opinion in Food Science, 11: 10–15.

Battilani, P., Logrieco, A. F., 2014: Global risk maps for mycotoxins in wheat and maize. In: Leslie, J. F., Logrieco, A. (eds.) Mycotoxin reduction in grain chains: a practical guide. Wiley-Blackwell, London, UK: 309–326.

Berthiller, F., Dall'Asta, C., Schuhmacher, R., Lemmens, M., Adam, G., Krska, R., 2005: Masked mycotoxins: Determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography-tandem mass spectrometry. Journal of Agricultural and Food Chemistry, 53: 3421–3425.

Calado, T., Venancio, A., Abrunhosa, L., 2014: Irradiation for mold and mycotoxin control: A review. Comprehensive Reviews in Food Science and Food Safety, 13: 1049–1061.

Degen, G. H., 2017: Mycotoxins in food. Occurrence, importance and health risk. Bundesgesundheitsblatt-Gesundheitsforschung-Gesundheitsschutz, 60: 745–756.

Homdork, S., Fehrmann, H., Beck, R., 2000: Influence of different storage conditions on the mycotoxin production and quality of Fusarium-infected wheat grain. Journal of Phytopathology-Phytopathologische Zeitschrift, 148: 7–15.

Kamala, A., Kimanya, M., Lachat, C., Jacxsens, L., Haesaert, G., Kolsteren, P., Ortiz, J., Tiisekwa, B., De Meulenaer, B., 2017: Risk of exposure to multiple mycotoxins from maize-based complementary foods in Tanzania. Journal of Agricultural and Food Chemistry, 65: 7106–7114.

Magan, N., Aldred, D., 2007: Post-harvest control strategies: Minimizing mycotoxins in the food chain. International Journal of Food Microbiology, 119: 131–139.

Mastanjević, K., Krstanović, V., Mastanjević, K., Šarkanj, B., 2018: Malting and brewing industries encounter *Fusarium* spp. related problems. Fermentation, 4: 3.

Nakajima, T., Yoshida, M., Kawada, N., 2008: Fungicide application timing on fusarium head blight and mycotoxin accumulation in barley in Japan. Cereal Research Communications, 36: 659–665.

Nielsen, K. F., Smedsgaard, J., 2003: Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography-UV-mass spectrometry methodology. Journal of Chromatography A, 1002: 111–136.

Oswald, I. P., Marin, D. E., Bouhet, S., Pinton, P., Taranu, I., Accensi, F., 2005: Immunotoxicological risk of mycotoxins for domestic animals. Food Additives and Contaminants, 22: 354–360.

Pascari, X., Ramos, A. J., Marin, S., Sanchis, V., 2018: Mycotoxins and beer. Impact of beer production process on mycotoxin contamination. A review. Food Research International, 103: 121–129.

Reverberi, M., Ricelli, A., Zjalic, S., Fabbri, A. A., Fanelli, C., 2010: Natural functions of mycotoxins and control of their biosynthesis in fungi. Applied Microbiology and Biotechnology, 87: 899–911.

Schmidt-Heydt, M., Stoll, D., Geisen, R., 2013: Fungicides effectively used for growth inhibition of several fungi could induce mycotoxin biosynthesis in toxigenic species. International Journal of Food Microbiology, 166: 407–412.

Sip, V., Chrpova, J., Veskrna, O., Bobkova, L., 2010: The impact of cultivar resistance and fungicide treatment on mycotoxin content in grain and yield losses caused by Fusarium Head Blight in wheat. Czech Journal of Genetics and Plant Breeding, 46: 21–26.

Skladanka, J., Nedelnik, J., Adam, V., Dolezal, P., Moravcova, H., Dohnal, V., 2011: Forage as a primary source of mycotoxins in animal diets. International Journal of Environmental Research and Public Health, 8: 37–50.

Stanciu, O., Juan, C., Miere, D., Loghin, F., Manes, J., 2017: Presence of enniatins and beauvericin in Romanian wheat samples: From raw material topProducts for direct human consumption. Toxins, 9: 189.

Vejdovszky, K., Hahn, K., Braun, D., Warth, B., Marko, D., 2017: Synergistic estrogenic effects of Fusarium and Alternaria mycotoxins in vitro. Archives of Toxicology, 91: 1447–1460.

Wolf-Hall, C. E., 2007: Mold and mycotoxin problems encountered during malting and brewing. International Journal of Food Microbiology, 119: 89–94.

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