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PRODUCTION OF T-2 TOXIN AND DEOXYNIVALENOL IN THE PRESENCE OF DIFFERENT DISINFECTANTS

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ABSTRACT

The aim of the work was to examine the effect of different disinfectants on production trichothecenes (especially of T-2 toxin and deoxynivalenol). Lipophilicity, chemical structure, the presence of bioactive groups and functional groups in their structure modifies biological activity and toxic potency of trichothecenes. For this reason, limits have been established designating maximum levels of mycotoxins in cereals while maintaining proper growing practices. Appropriate nutritive media were prepared with different concentration of tested disinfectants (Desanal A plus, ProCura spray and Guaa-Pool) and were inoculated using *Fusarium* strains. The density of *Fusarium* was 10^5 spores per mililitre. Nutrient media was cultivated at 15 °C and 25 °C for seven days. The strains of *Fusarium graminearum* CCM F-683 and *Fusarium* species (isolated from barley) produced quantities of deoxynivalenol. *Fusarium poae* CCM F-584 and *Fusarium* species (isolated from malthouse air) produced quantities of T-2 toxin. Desanal A plus prevented *Fusarium* growth and production of T-2 toxin and deoxynivalenol at the concentration 10%. It is an alkaline disinfectant on the basis of active chlorine and the surfactant that contains <5% of NaClO. ProCura spray at the concentration 0.6% proved to be very effective. This disinfectant contains 35% of propan-1-ol and 25% of propan-2-ol. Guaa-Pool at the concentration 0.004% proved to be very effective. It is a polymeric disinfectant with anion surface-acting agent and it contains <0.9% of polyhexamethylene guanidine hydrochloride and <0.2% of alkyl (C12-C16) dimethylbenzyl ammonium chloride. Lower contentration of disinfectants that not prevented growth of *Fusarium* caused higher production of T-2 toxin and deoxynivalenol. The contents of T-2 toxin and deoxynivalenol were analyzed by enzyme-linked immunosorbent assay (ELISA) using commercially produced kits (Agra Quant® Deoxynivalenol Test kit and Agra Quant® T-2 toxin Test kit). The experiment showed that the variability in the production of T-2 toxin and deoxynivalenol depended on the *Fusarium* strain used, concentration of disinfectants and temperature of cultivation.

Keywords: disinfectant; T-2 toxin; deoxynivalenol; *Fusarium*; ELISA

INTRODUCTION

Fusarium mycotoxins cause various diseases in humans and livestock. T-2 toxin and deoxynivalenol (trichothecene mycotoxins) produced by fungi of the genus *Fusarium* (*F.*) are considered the most common contaminants in feed and food in our climatic conditions. Contamination of cereals with mycotoxins produced by *Fusarium* species is a worldwide problem **(Vasatkova et al., 2009)**. *Fusarium* species contaminate the crops and embryonic tissue of seeds with spores during their growth and development **(Thammawong et al., 2011; Schollenberger et al., 2007)**. *Fusarium* species occure on the ear of cereals, stalks, roots or leaves of plants. Strains of *F. poae*, *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. nivale*, *F. sporotrichioides*, *F. cerealis*, and *F. equiseti* are often isolated. They are producers of a wide range of mycotoxins (e.g., trichothecene mycotoxins, zearalenones, fumonisins, moniliformin) **(Malíř a Ostrý, 2003; Gärtner et al., 2008; D´Mello et al., 1999; Malachová et al., 2010; Bottalico a Perrone, 2004)**. They influence reproduction, prevent maturation of oocytes, disrupt of protein synthesis and affect intracellular regulators. Mycotoxins are dangerous for humans because they have mutagenic, carcinogenic, teratogenic and immunosuppressive effects. The exposition to trichothecene mycotoxins can result in liver damage, damage to endocrine and nervous systems **(Crrepy, 2002; Hussein a Brasel, 2001; Pestka et al., 2004; Bryden, 2007; Wu et al., 2012)**.

Trichothecene mycotoxins (trichothecenes) are the largest group of mycotoxins occurring around the world. They can be produced by taxonomically dissimilar genera of fungi, such as *Fusarium*, *Cryptomela*, *Dendrodochium*, *Trichothecium*, *Hypocrea*, *Trichoderma*, *Phomosis*, *Cylindrocarpon*, *Stachybotrys* and *Myrothecium* **(Desjardins, 2006; Li et al., 2011)**. These are polycyclic sesquiterpenoids containing an 12,13-epoxy ring that is responsible for their toxicity. On the basis of the presence of or absence of characteristic functional groups they can be classified into four types A-D. Trichothecenes type B is different from the type A due to the presence of the carbonyl group at the C-8 position. Type A includes T-2 toxin (Figure 1) and HT-2 toxin. Type B is represented by deoxynivalenol (Figure 2) and nivalenol. In terms of toxicity trichothecene mycotoxins of type A are more dangerous than type B. Trichothecene mycotoxins of type C (crotocin, baccharin) have a second epoxy group between C-7 and C-8 or C-9 and C-10. Satratoxin and

roridin (type D) are characterized by a macrocyclic ring between C-4 and C-15 **(Monaci et al., 2011; Sudakin, 2003; Li et al., 2011; Delgado et al., 2010; Krska et al., 2011)**. Mycotoxin production can be affected by temperature, humidity, type of substrate, and applications of fertilizer and fungicidal agents **(Haidukowski et al., 2012; Hrubošová-Hrmová et al., 2011)**. Mold growth and production of mycotoxins can be avoided by proper choice of disinfectants. The problems in the use of disinfectants in food industry must be in the processing of food. If mycotoxins are already produced, they are usually not influenced by technological processing. Aflatoxins, ochratoxin A, deoxynivalenol, zearalenone, fumonisin and other are highly stable. Breweries worldwide had and still have problems with gushing when barley contains mycotoxins produced by *Fusarium* fungi. First step is to prevent the occurrence of *Fusarium* on barley by fungicides. Second step is preventing the occurrence using of different disinfectants in malt-houses **(Běláková et al., 2012; Suchý a Herzig, 2014)**.

The aim of this study was to determine the effect of different disinfectants on production of T-2 toxin and deoxynivalenol.

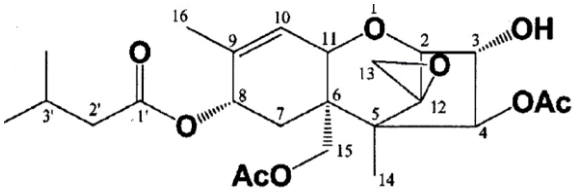

Figure 1 T-2 toxin **(Li et al., 2011)**

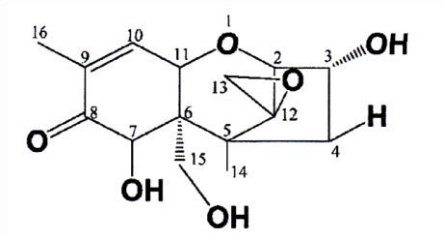

Figure 2 Deoxynivalenol **(Li et al., 2011)**

MATERIAL AND METHODOLOGY

Fusarium strains

Fusarium poae CCM F 584 and *Fusarium graminearum* CCM F 683 are from the Czech Collection of Microorganisms (Brno, Czech Republic). *Fusarium* species (*Fusarium* spp. 1) was isolated from malt-house air and *Fusarium* species (*Fusarium* spp. 2) was isolated from barley.

Cultivation media

MALT agar, DRBC agar, and potato-carrot agar (HiMedia, Mumbai, India) were used for isolation and identification of *Fusarium* from samples. Furthermore MALT agar was used for isolation of T-2 toxin and deoxynivalenol in the presence of various concentrations of disinfectants.

Disinfectants

Desanal A plus (Mica, Česká Třebová, Czech Republic)
• used concentrations: 3, 5, 7 and 10%
The concentration recommended by the manufacturer is 7-10%. It is an alkaline disinfectant on the basis of active chlorine and the surfactant that contains <5% of NaClO.

ProCura spray (Agrochem, Praha, Czech Republic)
• used concentrations: 0.2, 0.3, 0.4, 0.5 and 0.6%
The concentration recommended by the manufacturer is 1-2%. This disinfectant contains 35% of propan-1-ol and 25% of propan-2-ol.

Guaa-pool (Guapex, Brno, Czech Republic)
• used concentrations: 0.0005, 0.001, 0.002, 0.003 and 0.004%
The concentration recommended by the manufacturer is 0.0004-0.0012%. It is a polymeric disinfectant with anion surface-acting agent and it contains <0.9% of polyhexamethylene guanidine hydrochloride and <0.2% of alkyl (C12-C16) dimethylbenzyl ammonium chloride.

Cultivation of *Fusarium* in the presence of disinfectants

The monitoring of the *Fusarium* mycotoxins production in the presence of disinfectants was performed as follows: tested concentrations of different disinfectants were added into the Malt agar. This prepared nutrient medium was inoculated with 0.1 mL of *Fusarium* spores at a density of 10^6 spores per millilitre (*Fusarium poae* CCM F 584, *Fusarium graminearum* CCM F 683, *Fusarium* spp. 1, *Fusarium* spp. 2). The density of spores was determined using the Bürker chamber. Cultivation was carried out at temperatures of 15 °C and 25 °C for 7 days.

Isolation of T-2 toxin and DON

The content of the respective Petri dish after cultivation (20 g of sample) was mixed with 100 mL of acetonitrile : distilled water mixture (3:1) for 30 min at room temperature at 375 g on a digital shaker (Labnet, Edison, New Jersey, USA) **(Hlaváčková et al., 2012).** After the filtration of the obtained volume, 1 mL was purified using on EASI-EXTRACT T-2 HT-2 immunoaffinity column (R-Biopharm, Darmstadt, Germany) for the T-2 toxin and using DONtest HPLC (Vicam, Milford, Massachusetts, USA) for DON and evaporated to dryness. Prior to the actual analysis, the residue after evaporation was dissolved in 1 mL of distilled water.

Determination of T-2 toxin and DON

T-2 toxin and DON were analysed according to the instructions in Agra Quant® Deoxynivalenol Test kit (Romer Labs, Tulln, Austria, quantification limit: 250 $\mu g.kg^{-1}$) and Agra Quant® T-2 toxin Test kit (Romer Labs, Tulln, Austria, quantification limit: 75 $\mu g.kg^{-1}$). Samples containing the toxin below the limit of quantification were measured by the method of standard addition. The standard addition of T-2 toxin was 100 $\mu g.kg^{-1}$ and standard addition of DON was 300 $\mu g.kg^{-1}$. The absorption in the micro-wells was measured with a Multiskan RC ELISA reader (Ani Labsystems Oy, Vantaa, Finland) using 450 nm of

absorbance filter. All experiments were repeated three times.

RESULTS AND DISCUSSION

In research were created suitable conditions for the germination of spores and production of T-2 toxin and deoxynivalenol. Adding of disinfectants into nutrient media had prevent *Fusarium* growth and production of T-2 toxin and deoxynivalenol.

Production of T - 2 toxin was examined in *Fusarium poae* CCM F 584 and *Fusarium* spp. 1 - isolated from malt-house air. A content of T-2 toxin is mentioned in Table 1. The highest production of T - 2 toxin at 25 °C was shown by the tested strain *Fusarium* spp. 1 in the presence of 3% of Desanal A plus. T -2 toxin at 15 °C produced mainly *Fusarium poae* CCM F 584 cultivated in the presence of 5% Desanal A plus and 0.3% Procure star namely 84.6 ±0.3 to 85.9 ±0.6 $\mu g.kg^{-1}$. When comparing two different strains was found difference production of T -2 toxin depending on the strain and temperature of the cultivation. The difference in the incidence of *Fusarium* confirms psychrophilic strains that described the **Weidenbörner et al. (2001)**. As psychrophilic *Fusarium* can be considered strain of *Fusarium poae* CCM F 584 because the increase production of T - 2 toxin occurred more at 15 °C in comparison to 25 °C. **Kokkonen et al. (2010)** found that a water activity and temperature influence the production of mycotoxins. This was observed both in the production of T-2 toxin and deoxynivalenol in our experiment.

Production of deoxynivalenol was examined in *Fusarium graminearum* CCM F 683 and *Fusarium* spp. 2 - isolated from barley. The higher production of deoxynivalenol was observed in *Fusarium graminearum* CCM F 683 cultivated in the presence of 3% Desanal A plus at 25 °C (28.9 ±0.9 $\mu g.kg^{-1}$) and *Fusarium* spp. 2 cultivated in the presence of 7% Desanal A plus at 25 °C (27.9 ±1.6 $\mu g.kg^{-1}$). The experiment showed that the use of lower concentrations of Desanal A plus disinfectants then 10% not prevented growth of *Fusarium* and production of T-2 toxin and deoxynivalenol. It was also confirmed in the study of **Noske a Shearer (1985)**. **Reynolds et al. (2012)** recommended the using of low concentrations (2.4%) of NaClO for the reduction of fungi because sodium hypochlorite which is weakly alkaline (9-12 pH) can cause skin irritation **(Hostynek et al., 2006)**. In the presence of ProCura star *Fusarium graminearum* CCM F 683 was production of deoxynivalenol in the range of 11.9 ±0.9 $\mu g.kg^{-1}$ to 35.4 ±2.2 $\mu g.kg^{-1}$ and *Fusarium* spp. 2 was production of

Table 1 Production of T-2 toxin.

		T-2 toxin [$\mu g.kg^{-1}$] ±SD **			
Disinfectant	**Concentration (%)**	***Fusarium poae* CCM F 584**		***Fusarium* spp. 1***	
		25 °C	**15 °C**	**25 °C**	**15 °C**
Desanal A plus	3	59.4 ±0.9	77.6 ±0.5	82.7 ±0.3	69.4 ±1.4
Desanal A plus	5	56.7 ±0.5	84.6 ±0.3	80.0 ±1.7	0
Desanal A plus	7	0	37.1 ±1.1	0	0
Desanal A plus	10	0	0	0	0
Guaa-Pool	0.0005	23.2 ±1.3	31.6 ±0.8	39.8 ±0.3	52.1 ±0.6
Guaa-Pool	0.001	4.5 ±0.4	2.5 ±0.6	15.2 ±0.1	44.4 ±0.3
Guaa-Pool	0.002	18.9 ±0.7	20.8 ±0.9	43.3 ±0.6	37.0 ±0.2
Guaa-Pool	0.003	12.5 ±0.7	18.3 ±0.5	46.7 ±0.8	48.5 ±1.0
Guaa-Pool	0.004	0	0	0	0
ProCura star	0,2	9.2 ±0.1	39.7 ±0.4	61.6 ±0.8	34.5 ±0.8
ProCura star	0,3	21.0 ±0.5	85.9 ±0.6	44.2 ±0.5	0.8 ±0.6
ProCura star	0,4	31.3 ±1.7	0	45.2 ±1.9	3.6 ±0.1
ProCura star	0,5	25.7 ±0.2	0	0	0
ProCura star	0,6	0	0	0	0
without disinfectants	**0**	**13.6 ±0.9**	**93.7 ±1.2**	**71.6 ±0.9**	**13.0 ±2.3**

* *Fusarium* spp. 1 - isolated from malt-house air

** average value ±standard deviation

deoxynivalenol in the range of 24.6 ±1.9 μg.kg^{-1} to 38.1 ±2.0 μg.kg^{-1}. The 0.5% concentration of ProCura star proved to be very effective. **Suchomel et al. (2009)** found that alcoholic disinfectants containing mixtures of propan-1-ol and propan-2-ol at alcohol concentrations 70% or more fulfilled the required standard of antimicrobial efficacy. Quantity of deoxynivalenol produced by *Fusarium graminearum* CCM F 683 and *Fusarium* spp. 2 in the presence of Guaa-Pool is shown on Figure 3 and Figure 4. Guaa-Pool is a polymeric disinfectant with anion surface-acting agent. Quaternary ammonium compounds are widely used biocides with antimicrobial effect against a broad range of microorganisms. It is shown in the study of **Wessels a Ingmer (2013)**.

Our experiment showed that low concentrations of disinfectants did not prevent production of mycotoxins. It was found that using low concentrations of disinfectant can cause increased production of toxins. This behavior of fungi has already been described when cultivated in the presence of fungicides **Havlová et al. (2006)** and **Heier et al. (2005)**. Improper application of disinfectants may act as a stress factor stimulating the production of mycotoxins, and therefore we must comply with all instructions given by the manufacturer.

CONCLUSION

The results of the study show that the most effective disinfectant for reduction of production of T-2 toxin and deoxynivalenol was Desanal A plus and ProCura star. Guaa-Pool at the concentration 0.004% proved to be very effective. It is higher concentration of disinfectant than recommended by manufacturer for full fungicidal activity. Lower contentration of disinfectants that not prevented growth of Fusarium caused higher production of T-2 toxin

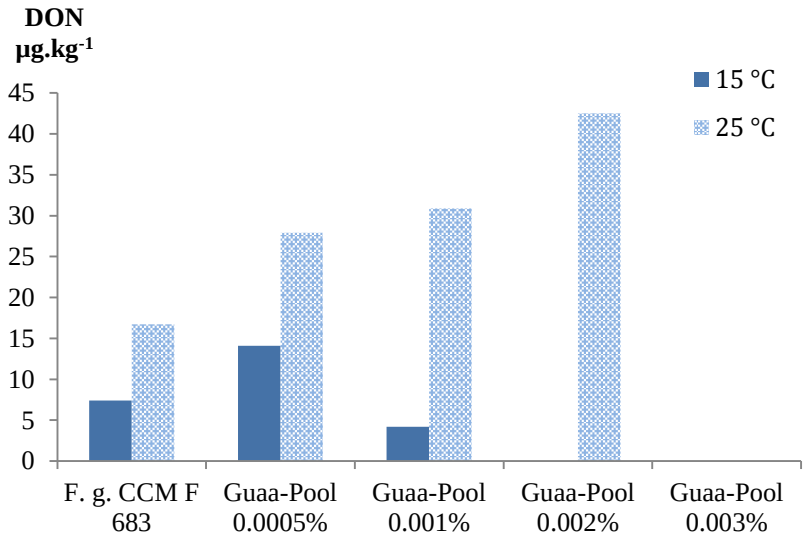

Figure 3 Production of deoxynivalenol, *Fusarium graminearum* CCM F 683, cultivation for 7 days.

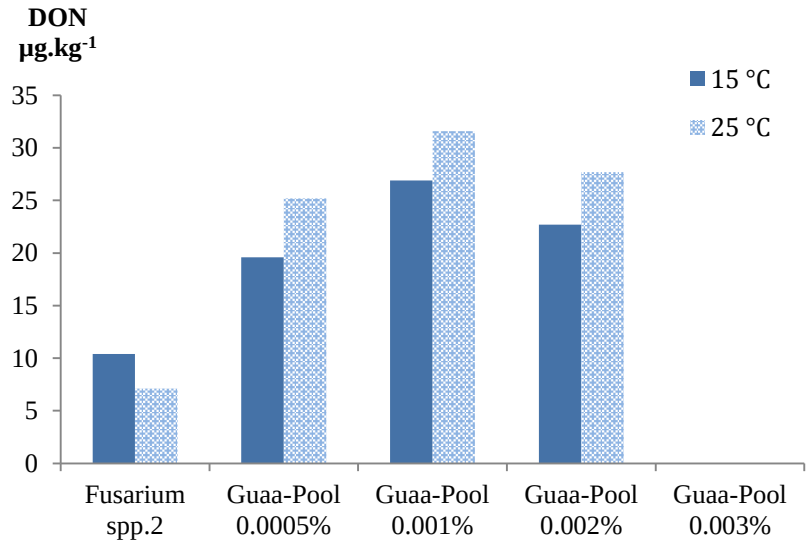

Figure 4 Production of deoxynivalenol, *Fusarium* spp. 2, cultivation for 7 days.

and deoxynivalenol. The results indicate considerable variability of individual strains in the production of T-2 toxin and deoxynivalenol. It depended on the *Fusarium* strain used, concentration of disinfectants and temperature of cultivation.

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