

## OVERWINTERING OF FODDER BARLEY ARTIFICIALLY INFECTED WITH FUMONISIN

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### Abstract

Production of *Fusarium* mycotoxins is an important problem of grain samples including barley and wheat in most producing areas worldwide. The study describes a new barley testing methodology based on various concentrations of artificially inoculated healthy and damaged seeds to assess the potential of passed mycotoxins. Eight variants presented in this survey were carry out in three repetitions (pots). Fumonisin levels were monitored before and after artificially inoculated barley grains by ELISA method. In order to determine the resistance of barley, the investigated grains were inoculated with spike solution of different concentrations including 0.666, 2 and 6 mg/l fumonisin. In the damaged samples inoculated with the highest concentration was detected the fumonisin's molecules to passed in low quantity. After overwintering in the same samples the presence of fumonisins was detected of very low concentration of 0.347 mg/kg. This evaluation provide important information about the resistance of barley grains considering different levels of fumonisin contamination. The results show that in seeds contaminated with a concentration of 6 mg/l or higher, there is a possibility that the mycotoxin can pass into the seeds and even infect the crop after wintering.

**Key words:** barley, crop, fumonisin, grains, mycotoxins

### Introduction

*Fusarium* mycotoxins are fungus metabolites produced by different *Fusarium* spp. on the harvested small-grain cereals and straw which in high concentrations represent a risk to human and animal health. Although *Fusarium* species may cause infections in humans and animals, they are mainly soil-born pathogens affecting plants and causing devastating crop disease and yield loss in some key crops such as barley, wheat, maize, cotton, bananas, etc. *Fusarium* is one of the largest genus filamentous fungi, represented by more than a thousand species with a number of continuously emerging new races and isolates. A complex of toxigenic and nontoxigenic *Fusarium* species has been isolated from cereals (wheat, barley, and oat) with symptoms of *Fusarium* head blight (FHB), a major disease of small-grain cereals (grains) that reduces seed quality and yield (Pestka, 2010; Zinedine et al., 2007).

The influence of climatic conditions on the accumulation of mycotoxins most probably is an indirect factor, influencing primarily fungal growth (Popovski and Celar, 2013). However, disease development depends on the substrate, *Fusarium* species present and individual metabolite since every species of *Fusarium* head blight (FHB) complex have different environmental demands. Mycotoxins produced by *Fusarium* species pose a threat to both, animal and human health. One of the most abundant mycotoxins and therefore most frequently contaminating grains and food products is fumonisin (Mielniczuk et al., 2020). Fumonisin are a group of 15 structurally related mycotoxins, and three of them (FB1, FB2 and FB3) can cause serious health

problems in animals such as equine leukoencephalomalacia in horses, pulmonary edema in swine, or kidney cancer in rats or mice (Riley, R. T. and A. H. Merrill, 2019).

Knowledge about the most influencing cultivation measures such as crop rotation, choice of the variety and tillage is important to reduce the risk of a *Fusarium* infection in barley and was assessed during the recent years (Schöneberg et al. 2016). In addition, data about the most susceptible growth stages and favourable climatic conditions, which were shown to affect the infection with *Fusarium* spp., are necessary to predict the occurrence in a particular field (Cowger et al. 2009; Brustolin et al. 2013).

Considering the findings of harmful mycotoxin effects on human and animal health, the aim of this study was to estimate the effect of different concentrations of fumonisins in artificially inoculated barley during overwintering.

## **Materials and methods**

### **Plant material and soil trials**

The study was conducted in vegetative season 2022/2023 at location Kostinbrod, Bulgaria. The soil type used for the vessel experiment is alluvial. An agrochemical analysis was carried out, which includes: pH, total mineral nitrogen - according to Bremner and Kine's model, phosphorus and potassium - according to P. Ivanov's method, humus - according to Tyurin's method, mechanical composition - according to the pyrophosphate method, determination of the number of microorganisms from main groups (bacteria, microscopic fungi, actinomycetes and cellulose-decomposing microorganisms) - by the method of decimal dilutions.

### **Micotoxin analysis**

Barley, before being sown in a vessel experiment, was examined for the presence of fumonisins. Samples analyses: 5 g of ground samples were mixed and processed using 70% methanol (Valerus Ltd, Bulgaria) as solvent for extraction. The filtered (Whatman No 1, Merck) samples were screened by the Enzyme-Linked Immunosorbent Assay (ELISA) method. A commercial kit (R-Biopharm, Germany) was used and the samples were prepared according to the instructions of the manufacturer. The measurement was made at 450 nm. The values calculated for the standards were entered into the Ridawin program, Computer Systems (ELx800 Universal Microplate Reader, BIOTEK® Instruments, Inc., USA).

Chemical analysis of the barley samples was also performed.

### **Inoculation Procedure**

Healthy and mechanically damaged barley seeds variety "Veslets" were used in this survey. The samples intended for the vessel experiment were artificially inoculated with fumonisin by spiking with different concentrations of 0.666, 2 and 6 mg/l and stayed in spiking solution overnight.

Scheme of the experiment:

1. Control – healthy seeds untreated with fumonisin
2. Healthy seeds treated with fumonisin at concentration of 0.660 mg/l
3. Healthy seeds treated with fumonisin at concentration of 2 mg/l
4. Healthy seeds treated with fumonisin at concentration 6 mg/l
5. Mechanically damaged seeds not treated with fumonisin
6. Mechanically damaged seeds treated with fumonisin at concentration of 0.660 mg/l
7. Mechanically damaged seeds treated with fumonisin at concentration of 2 mg/l
8. Mechanically damaged seeds treated with fumonisin at concentration of 6 mg/l

Eight variants were presented in three repetitions (pots). Three grains of barley are sown in each pot.

## Results and Discussion

The presence of fumonisins in the investigated barley samples was analyzed by ELISA method. Healthy and mechanically damaged grains were artificially infected with various concentrations of fumonisin - 0.666, 2 and 6 mg/l and after sown in eight pots. With this survey for first time we evaluated how much quantity of the spiked mycotoxin is passed and contaminated the grains and what is the result after overwintering concerning the crop. The results showed that in the both types of treated samples, grains inoculation had no or little effect on germination and crop. This was especially observed in the damaged seeds infected with the highest concentration of 6 mg/l, figure 1.

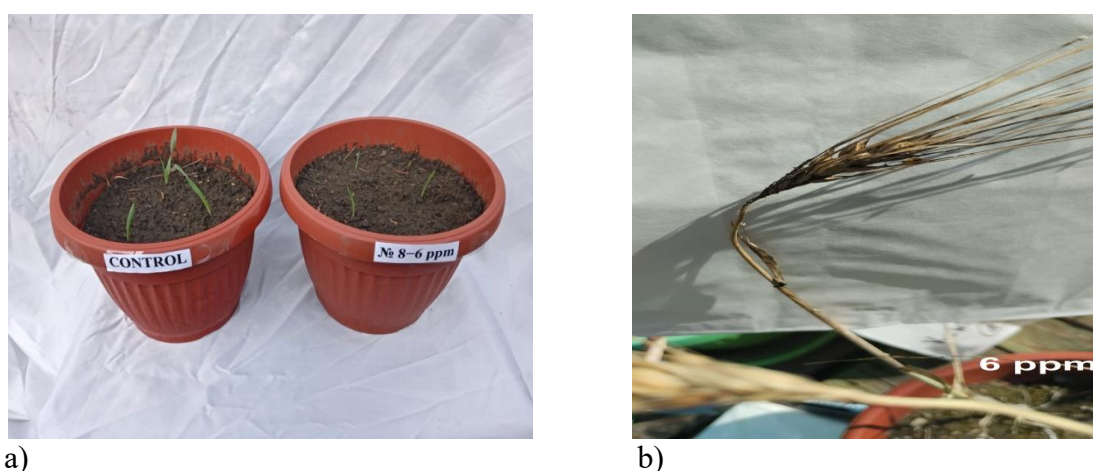


Figure 1. a) Germination of the control and artificially inoculated damaged barley grains with 6 mg/l fumonisin; b) crop of the damaged grains inoculated with 6 mg/l fumonisin

It should be noted that only at this concentration was detected the presence of fumonisin in seeds measured after spiking procedure. Probably this contamination influenced on the germination and the growth of the grains.

Similar results were found by Koch et al. (2020) where they inoculated maize kernels and found no or only a small effect on plant stand.

Continuing with the evaluation of the spiked mycotoxin after overwintering the detected value in the crop was 0.347 mg/kg. It has to be mentioned that this result was found in the mechanically damaged seeds, initially inoculated with 6 mg/l fumonisin. On the other hand the germination was also influenced in mechanically damaged barley grains artificially contaminated with lower concentration of 2 mg/l fumonisin. Probably this concentration also has an effect on the germination and plant growth. So, we found that various concentrations have different influence on the germination during overwintering, table 1.

The results show that for the concentration of 0.666 mg/l the damaged barley grains show resistance. Compare with artificially infected with *Fusarium* fungi wheat samples in a study performed by Sunic et al. (2021) where they found the genetic resistance considering mycotoxin accumulation in epidemic conditions. The FHB evaluations provide important information about the genetic resistance of wheat varieties, as well as risk assessment considering mycotoxin accumulation in epidemic conditions.

Table 1. Germination (%) in healthy and damaged barley grains inoculated by different concentrations of fumonisin

| Kind of samples                    | Autumn           |               | Spring           |               |
|------------------------------------|------------------|---------------|------------------|---------------|
|                                    | Number of plants | % germination | Number of plants | % germination |
| Healthy (not contaminated) control | 8                | 0.89          | 8                | 100           |
| H-0.666 mg/l                       | 9                | 100           | 8                | 89            |
| H-2 mg/l                           | 9                | 100           | 9                | 100           |
| H- 6 mg/l                          | 9                | 100           | 9                | 100           |
| D (not contaminated)               | 8                | 0.89          | 8                | 100           |
| D- 0.666 mg/l                      | 9                | 100           | 9                | 100           |
| D-2 mg/l                           | 8                | 0.89          | 7                | 88            |
| D-6 mg/l                           | 9                | 100           | 7                | 78            |

It has to be mentioned that the lowest percentage during the spring germination was observed of overwintering plants in the variant with damaged seeds inoculated with 6 mg/l fumonisin. Better was the situation concerning overwintering with damaged seeds at a concentration of 2 mg/l and for the variant with healthy seeds treated with 0.666 mg/l. In the other variants, 100% of the plants overwintered. Obviously there are other factors which influenced on the overwintering. Actually a tendency was found for artificially contaminated damaged seeds to have poorer germination than healthy seeds, figure 2.

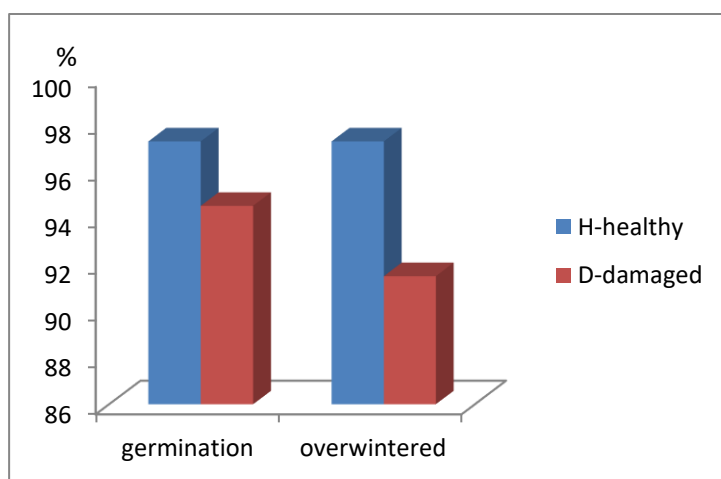


Figure 2. Germination and overwintering (%) of healthy and damaged seeds

From the data presented in table 2, it can be seen that there is some relationship between the concentration of spike solution and the germination of the plants afterwards. The highest mean brotherhood was found in the control variant (whole seeds not treated with fumonisin). The lowest brotherhood was recorded in the variants with damaged seeds at the two higher concentrations at 2 mg/l and 6 mg/l respectively (variants 7 and 8).

Table 2. Dependence between different concentrations of fumonisin and brotherhood

| Kind of samples                    | Spring           |                       |                  |                |
|------------------------------------|------------------|-----------------------|------------------|----------------|
|                                    | Number of plants | Number of brotherhood | Mean brotherhood | Number of ears |
| Healthy (not contaminated) control | 8                | 17                    | 12.5             | 9              |
| H-0.666 mg/l                       | 8                | 14                    | 11               | 12             |
| H-2 mg/l                           | 9                | 14                    | 11.5             | 14             |
| H- 6 mg/l                          | 9                | 13                    | 11               | 12             |
| Damaged (not contaminated)         | 8                | 9                     | 8.5              | 9              |
| D- 0.666 mg/l                      | 9                | 13                    | 11               | 10             |
| D-2 mg/l                           | 7                | 9                     | 8                | 9              |
| D-6 mg/l                           | 7                | 9                     | 8                | 9              |

It should be noted that an even more marked difference between the two types of seeds (whole and damaged) is observed in their ability to overwinter.

## Conclusions

Considering the findings of harmful mycotoxin effects on human and animal health, the aim of this study was to estimate the effect on different mycotoxin levels in artificially inoculated barley used for sowing. It is of great importance to investigate the toxicity, as well as consequently regulate maximal allowed concentration for specific feed. Furthermore, possible synergistic effects between certain metabolites need to be investigated more closely as they could interact together thus giving total toxicity.

With this survey we determined at what concentration the presence of fumonisins in the seeds could directly affect their germination and the ability of plants to overwinter. Various concentrations influenced in different way on plant germination. The inoculated mechanically damaged seeds show low germination and as well as low possibility for overwintering. It is very important for farmers to know that the seeds contaminated with fumonisin at a concentration of 6 mg/l or higher should not be used for sowing because of economic losses.

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