https://doi.org/10.61308/WBVH9176

Development of barley after artificial inoculation with different concentrations fumonisin

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Citation: Nikolov, A., Sertova, N. & Ignatova, M. (2026). Development of barley after artificial inoculation with different concentrations fumonisin. *Bulgarian Journal of Animal Husbandry, 63*(1), 47-53

Abstract: Barley is a favorable substrate for the development of fungal diseases, such as fungi of the genus *Fusarium* spp. They produce numerous toxic metabolites, one of which are fumonisins. Theaimofthestudywas toinvestigatetheimpactonthedevelopmentandyieldof barleyafterartificialinoculationofhealthyandmechanically-damagedseedsforsowingwithfumonisinatconcentrationsof 1 mg/l, 3 mg/l and 9 mg/l. The trial experiment with barley variety „Veslets“ was conducted in the 2023/2024 season. With the carried out investigations, we found that healthy barley seeds artificially contaminated with fumonisin at a concentration of 9 mg/l have impaired germination and overwintering. In mechanically damaged seeds with the same concentration, germination and overwintering of the seeds are compromised. Fumonisin has a negative impact on the yield of barley grain, with the most significant impact at a concentration of 9 mg/l. Barley plants sprouted from healthy seeds have significantly higher average height, average weight and yield compared to plants sprouted from mechanically damaged seeds.

Keywords: barley; fumonisin; artificial inoculation

INTRODUCTION

One of the main tasks in the science of animal nutrition is related to protecting them from diseases caused by the consumption of feed contaminated with mycotoxins.Fungal species of the genus *Fusarium* spp. are one of the most widespread causative agents of diseases in cereal crops and cause damage to plants throughout the growing season, especially in regions with a humid climate. Fumonisins are secondary toxic metabolites produced in cereal crops by pathogenic fungi of the genus *Fusarium* spp. (Oswald et al., 2005). They are the most common contaminants affecting small-grain crops, which depending on the concentration of contamination, lead to plant diseases, and in higher concentrations pose a risk to human and animal health.

Fusarium wilt in cereals leads to complete crop damage and under suitable weather conditions can cause losses of up to 100% (Mesterhazy, 1997; Desheva and Chavdarov, 2016). It causes significant damage to producers, as it most often manifests itself as an epidemic on plants, for example in 1988 in Germany (Ellnerm, 1999), Hungary (Ruckenbauer et al., 2000). The fungus is capable of persistent asymptomatic infections (Bacon and Hinton, 1996), which is of concern due to the impact on control strategies. Kirdoglo (2004) reports that in two-row barley varieties, hidden losses are 5-6 times higher than direct losses, and in multi-row barley - 3 times higher. The level of hidden losses is determined by many factors, such as reduced field germination, reduced productive tillering, and to a greater extent damage from other diseases. Annual yield losses can reach up to 30%.

Fusarium wilt is a naturally serious disease of wheat and barley. Infection is initiated after the ear has emerged from the flag leaf sheath in the stages of seed development (Bushnell et al. 2003).

Regarding the artificial infection of barley, there are not much studies, mainly some of them are related to malting barley.

In this regard, Schöneberg et al. (2018) conducted a study in which artificial infection of feed and malting barley with fungi of the genus*Fusarium* spp. The influence of different temperatures (10 °C, 15 °C, 20 °C) and different durations (4 h, 8 h, 12 h) at 99% relative humidity was studied in a climate chamber. It was found that when treating fodder barley at a temperature of 15 °C, up to three times higher artificial contamination with mycotoxins of the genus *Fusarium* spp. (deoxynivalenol) was detected compared to treatment at 10 °C and 20 °C.

The aim of the study was to investigate the influence of artificial inoculationwith different concentrations of fumonisin on the development and yield of barley.

MATERIAL AND METHODS

The trial with barley variety "Veslets" was conducted in the 2023/2024 season on the territory of the Institute of Animal Sciences - Kostinbrod.

Mycotoxicological analysis

Before sowing, the barley was tested for the presence of fumonisins.

Sample preparation for analysis: 5 g of ground samples were mixed and processed using 70% methanol (Valerus Ltd., Bulgaria) as an extraction solvent. The filtered (Whatman No 1, Merck) samples were tested using the enzyme-linked immunosorbent assay (ELISA) method. A kit from R-Biopharm, Germany was used and the samples were prepared according to the manufacturer's instructions. Measurements were 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).

Artificial infection procedure

The presence of fumonisin was not detected in the tested samples. After that, the seeds were artificially infected with different concentrations of fumonisin - 1; 3 and 9 mg/l and then sown according to the following scheme:

1. Control – healthy seeds not infected with fumonisin;
2. Healthy seeds infected with fumonisin at a concentration of 1 mg/l;
3. Healthy seeds infected with fumonisin at a concentration of 3 mg/l;
4. Healthy seeds infected with fumonisin at a concentration of 9 mg/l;
5. Mechanically damaged seeds not infected with fumonisin;
6. Mechanically damaged seeds infected with fumonisin at a concentration of 1 mg/l;
7. Mechanically damaged seeds infected with fumonisin at a concentration of 3 mg/l;
8. Mechanically damaged seeds infected with fumonisin at a concentration of 9 mg/l.

These eight variants are presented in three replications (pots). Three barley grains are sown in each pot. The crop was sown on 11.10.2023 and harvested on 03.07.2024.

The statistical processing of the data was carried out using the software product Statgraphics 19. The method used to distinguish the mean values is the Fisher least significant difference (LSD) procedure. With this method, there is a 5.0% risk of seeing that each pair of variants is significantly different when the actual difference is equal to 0.

RESULTS AND DISCUSSION

With this study, we evaluated the resistance of barley in relation to how the added mycotoxin changed the development of the studied crop and at what fumonisin contamination the feed grains should not be used for sowing, due to subsequent economic losses.

The results show that in both types of treated samples (healthy and mechanically damaged) the inoculation of the grains has some effect on germination and overwintering (Table 1). This is observed especially in mechanically damaged seeds, infected with the highest concentration of fumonisin - 9 mg/l, where the low-

est germination percentage was recorded, only 11%. On the other hand, germination was also affected, but to a lesser extent, again in mechanically damaged barley grains, artificially infected with the lower concentrations of 1 mg/l and 3 mg/l fumonisin. It is likely that these concentrations also have an effect on germination and plant growth.

It should be noted that only at concentration of 9 mg/l after artificial inoculation of mechanically damaged barley grains was the presence of fumonisin (1.487 mg/l) penetrated into the seeds before they were sown. This contamination probably affected both germination and overwintering of the sown grains.

It should be noted that in the variant with mechanically damaged seeds with a concentration of 9 mg/l there were no overwintered plants. This gives reason to believe that this concentration of fumonisin does not allow plants to develop their biological potential for survival at low temperatures. The overwintering situation was better in mechanically damaged seeds with a concentration of 1 mg/l, 3 mg/l and in mechanically damaged seeds not infected with fumonisin. In the variants with healthy seeds not infected with fumonisin and in those with a concentration of 3 mg/l all plants overwintered. We can also add the variant with 1 mg/l to this group, despite the slightly lower percentage of overwintered plants (89%). An unsatisfactory result of 50% of overwintered plants was registered in the variant with 9 mg/l. In fact, a tendency was found for artificially infected damaged seeds to have worse germination and ability to overwinter than healthy ones.

In general, it can be said that the grain yield is reciprocal to the number of overwintered plants, which is understandable, but it should still be noted that the highest yield in the healthy uninfected plants variant is 5.2 g (Table 2).In the variant with mechanically damaged seeds infected with 9 mg/l fumonisin in the absence of overwintered plants, no yield was recorded. In this regard, it is

Table 2. Yield of artificially inoculated healthy and mechanically damaged seeds

Variants	Yield, g
Healthy uninfected (Control)	5.2
Healthy - 1 mg/l	4.7
Healthy - 3 mg/l	4.6
Healthy - 9 mg/l	2.3
Damaged uninfected	4.7
Damaged - 1 mg/l	3.5
Damaged - 3 mg/l	3.6
Damaged - 9 mg/l	-

Source: Authors' own elaboration

Table 1. Germination and overwintering of plants from healthy and damaged barley grains inoculated with different concentrations of fumonisin

Variants	Autumn		Spring	
	Number of plants	germination (%)	Number of plants	wintered (%)
Healthy uninfected (Control)	9	100	9	100
Healthy - 1 mg/l	9	100	8	89
Healthy - 3 mg/l	7	77	7	100
Healthy - 9 mg/l	6	67	3	50
Damaged uninfected	8	89	7	87
Damaged - 1 mg/l	8	89	6	75
Damaged - 3 mg/l	8	89	6	75
Damaged - 9 mg/l	1	11	-	-

Source: Authors' own elaboration

important to note that by the mycotoxicological analysis was not detect the presence of fumonisin in healthy uninfected grains. The harvest from the other variants was not examined due to the low yield, which turned out to be insufficient (<5 g) to perform the analysis.

Table 3. Multiple Range Tests for Yield by Treatments
Method: 95.0 percent LSD

Treatments	*Count*	*Mean*	*Homogeneous Groups*
Damaged - 9 mg/l	3	0	A
Healthy - 9 mg/l	3	2.3	B
Damaged - 1 mg/l	3	3.5	C
Damaged - 3 mg/l	3	3.6	C
Healthy - 3 mg/l	3	4.6	D
Damaged uninfected	3	4.7	D
Healthy - 1 mg/l	3	4.7	D
Healthy uninfected (Control)	3	5.2	E

Contrast	*Sig.*	*Difference*
Damaged - 1 mg/l - Damaged - 3 mg/l		-0.1
Damaged - 1 mg/l - Damaged - 9 mg/l	*	3.5
Damaged - 1 mg/l - Damaged uninfected	*	-1.2
Damaged - 1 mg/l - Healthy - 1 mg/l	*	-1.2
Damaged - 1 mg/l - Healthy - 3 mg/l	*	-1.1
Damaged - 1 mg/l - Healthy - 9 mg/l	*	1.2
Damaged - 1 mg/l - Healthy uninfected (Control)	*	-1.7
Damaged - 3 mg/l - Damaged - 9 mg/l	*	3.6
Damaged - 3 mg/l - Damaged uninfected	*	-1.1
Damaged - 3 mg/l - Healthy - 1 mg/l	*	-1.1
Damaged - 3 mg/l - Healthy - 3 mg/l	*	-1.0
Damaged - 3 mg/l - Healthy - 9 mg/l	*	1.3
Damaged - 3 mg/l - Healthy uninfected (Control)	*	-1.6
Damaged - 9 mg/l - Damaged uninfected	*	-4.7
Damaged - 9 mg/l - Healthy - 1 mg/l	*	-4.7
Damaged - 9 mg/l - Healthy - 3 mg/l	*	-4.6
Damaged - 9 mg/l - Healthy - 9 mg/l	*	-2.3
Damaged - 9 mg/l - Healthy uninfected (Control)	*	-5.2
Damaged uninfected - Healthy - 1 mg/l		0
Damaged uninfected - Healthy - 3 mg/l		0.1
Damaged uninfected - Healthy - 9 mg/l	*	2.4
Damaged uninfected - Healthy uninfected (Control)	*	-0.5
Healthy - 1 mg/l - Healthy - 3 mg/l		0.1
Healthy - 1 mg/l - Healthy - 9 mg/l	*	2.4
Healthy - 1 mg/l - Healthy uninfected (Control)	*	-0.5
Healthy - 3 mg/l - Healthy - 9 mg/l	*	2.3
Healthy - 3 mg/l - Healthy uninfected (Control)	*	-0.6
Healthy - 9 mg/l - Healthy uninfected (Control)	*	-2.9

**denotes a statistically significant difference.*

Source: Authors' own elaboration

Table 3 applies a multiple comparison procedure to determine which variants differ significantly from other variants. The lower half of the result shows the calculated difference between each pair of means. Up to 24 pairs are marked with an asterisk, indicating that these pairs show statistically significant differences at the 95.0% confidence level. At the top of the page, 5 homogeneous groups are identified using columns of letters. In each column, levels containing the same letter form a group of means within which there are no statistically significant differences. The control variant with healthy uninfected seeds is distinguished as a separate homogeneous group (E), which statistically proves that fumonisin affects barley yield to different degrees negatively. This is most noticeable in the two variants infected with 9 mg/l fumonisin (healthy and damaged seeds), which also formed two separate homogeneous groups (A) and (B), differing from each other only in that in homogeneous group (B) the yield is completely compromised.

The highest average plant height was recorded in the control variant of 58.3 cm (Fig. 1). In second place in terms of average height is the variant with damaged uninfected plants (52.7 cm.). These are the two variants in which the seeds are not infected with fumonisin and are expected to show the best results. In the other variants, both in healthy and damaged seeds, there is a tendency for the plant height to decrease with increasing fumonisin concentration, with the lowest average height being recorded in the variant with healthy seeds and a fumonisin concentration of 9 mg/l.

Regarding the average plant weight, the distribution by variants is similar to that of the distribution by average height (Fig. 2). There is a difference in the second place, where it is occupied by the variant with healthy seeds infected with 1 mg/l. A clearly expressed highest average weight is observed in the control variant (3.4 g), and the lowest (0.6 g) is registered in the variant with damaged seeds withfumonisin concentration of 3 mg/l.

Nowadays the invetsigations connected with artificialy innoculated barley with fungi are mainly associated with malting barley.

For instance, according to Biehl et al. (2025), in beer production interactions between primary

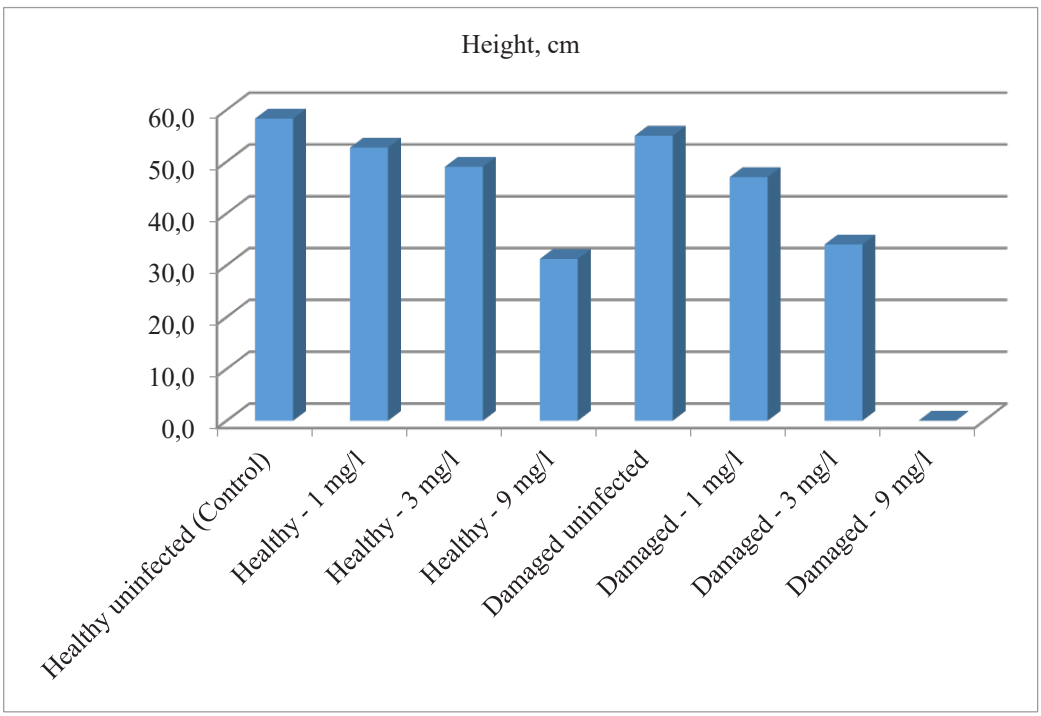


Figure 1. Average height of barley plants

Source: Authors' own elaboration

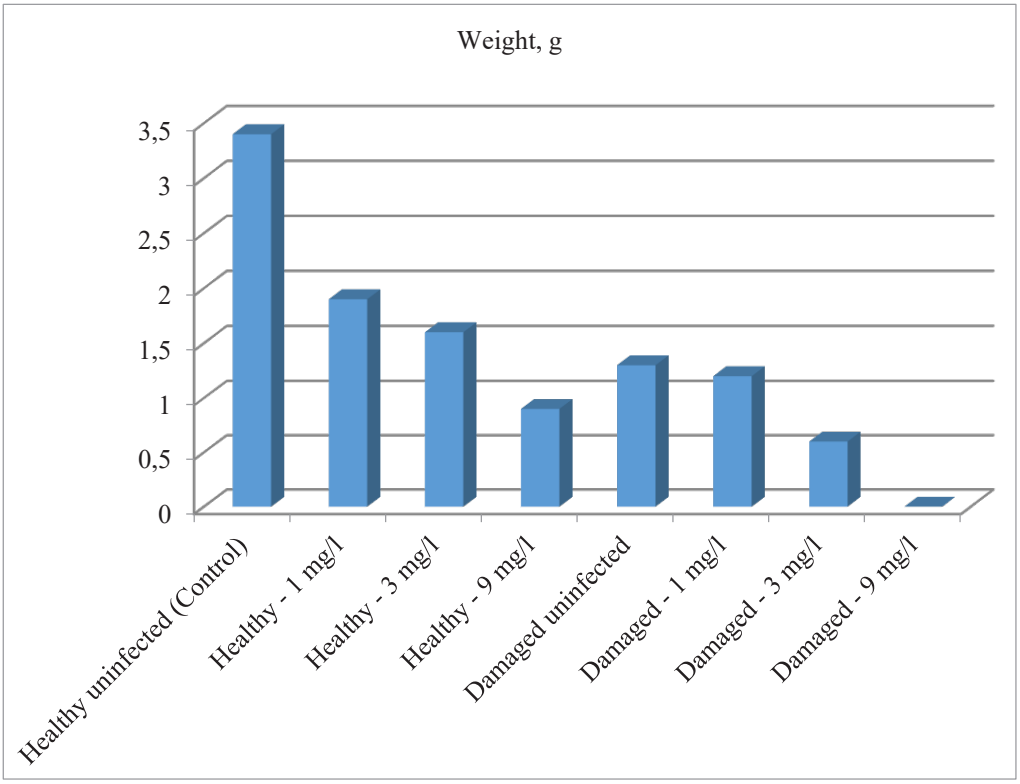


Figure 2. Average weight of barley plants

Source: Authors' own elaboration

fungal metabolites and secondary modified mycotoxins in barley, malt and beer are possible. In this regard, barley malt was inoculated with *F. Culmorum*spp. Mycotoxins were quantified during the malting process and compared with fungal DNA content. In this regard it is important to know the dynamics of mycotoxins in malt production, which helps to optimize conditions to minimize toxin levels in the final malt products.

Artificial inoculation allows researchers to track low levels of natural infection, thus allowing the detection of effects that would otherwise be unmeasurable.

CONCLUSION

Healthy barley seeds artificially contaminated with fumonisin at a concentration of 9 mg/l have impaired germination and overwintering. In mechanically damaged seeds with the same concentration, germination and overwintering of the seeds is compromised.

Fumonisin has a negative impact on barley grain yield, with the most significant impact at a concentration of 9 mg/l.

Barley plants germinated from healthy seeds have significantly higher average height, average weight and yield compared to plants germinated from mechanically damaged seeds.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Received: **November21, 2025**; *Approved*: **January26, 2026;** *Published:* **February, 2026**