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Real and statistical processing of barley yield data naturally contaminated with fusariotoxin

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Abstract: Fumonisins are produced by fungi of the genus *Fusarium* spp., which are widely spread out in the nature. They are produced during grain growth, ripening and harvesting and are known as "field mycotoxins". *Fusarium* species can produce compounds that are toxic to animals and humans. In this study, the development of *Fusarium* toxins was determined in healthy and mechanically damaged barley grains treated and untreated with fungicide, respectively, under natural contamination. It was found that healthy kernels treated with fungicide had the highest yield of 6700 $kg.ha^{-1}$ followed by the yield of damaged kernels treated with fungicide. The lowest yield of 4850 $kg.ha^{-1}$ was reported for the damaged and untreated barley grains. The two treatments whose plants were treated with fungicide had a higher yield than their counterparts, i.e. the plants not treated with fungicide. In confirmation of this by mycotoxicological analysis was established the presence of fumonisin 0.234 $mg.kg^{-1}$ in healthy seeds untreated with fungicide and 0.274 $mg.kg^{-1}$ in mechanically damaged untreated seeds. Fumonisin concentrations were measured by using Enzyme-Linked Immunosorbent Assay (ELISA) method. The used approach in this study shows that fungicide treatments effectively reduce mycotoxin levels in barley and increase grain yield. The use of fungicides should be combined with other infection mitigation strategies, such as avoiding mechanically damaged grains, which are a suitable substrate for mycotoxins production. These measures will help to improve food and feed safety.

Keywords: barley; yield; fungicide; mycotoxins

INTORDUCTION

During the growing season, plants are at risk from pathogenic microorganisms (viruses, bacteria, fungi), Havlova et al. (2006). The genus *Fusarium* is among the largest fungal genera consisting of pathogenic and non-pathogenic species (Zubi et al., 2021).

Protection of barley grain against contamination by fungi such as *Fusarium* spp., is of principal importance. Fungicides applied immediately after full heading of spring barley is one method of direct protection. Chemical protection against the attack of barley by *Fusarium* spp. is much more complicated than protection against foliar diseases. The fungicide should only be applied after full heading of the ear. Many investigations have been carried out in different parts of the world on the management of barley seed borne diseases. Diseases are known to reduce seed size and thus adversely affect seed quality (Havlova et al., 2006).

Recently, considerable attention has been devoted to diseases occurring in the ear, the most significant of which are those caused by fungi of the species *Fusarium*. Application of fungicides may protect the plant against *Fusarium* spp. pathogens and prevent damage to the grain. Many of these pathogens can be seed-or soilborne, but relatively little research has been done on the effects of seedborne fungal infection of barley and its management with fungicidal seed treatments (Seepe et al., 2021).

Harmful effects of seed diseases can be minimized by cultural practices and seed-applied fungicides. Good cultural practices, especially sowing seeds in soil that is warm, dry and free of chemical stressors, can reduce seed and seedling diseases (Solorzano and Malvick, 2011). Barley seeds are often treated with fungicides to reduce seed diseases.

Poor quality seeds will result in low germination in the field and seedlings that are less tolerant to abiotic stress. Fungicides are widely used in arable agriculture to reduce disease burden and its impact on yields and quality, yet the effect of fungicides on yield is far from clear. While some field studies show overall increases in yield (Stetkiewicz et al., 2019).

In order to target and reduce fungicide inputs, while maintaining high yields, it is necessary to understand under what conditions (i.e. weather, varietal resistance level, previous crop, etc.) fungicide application impacts yields. Applications can then be tailored to situations where a yield increase is likely to occur, and eschewed when yield is unlikely to be impacted.

Little is known about the effects of spray treatments on the growth and yield of barley grown from infection with fungal pathogens. The purpose of this study was to determine the effect of application of fungicide with an active substance of 250 g/l tebuconazole on barley yield obtained from healthy and mechanically damaged grains. Statistical analysis is also presented to assess the potential impact of fungicide treatment.

MATERIALS AND METHODS

Plant material and field trials

The study was carried out in vegetative season 2022/2023 at location Kostinbrod, Institute of Animal Science, Bulgaria. The soil type is Leached Vertisol with a heavy soil texture with a neutral to slightly alkaline soil reaction. In the 0-20 cm layer, the soil has a high phosphorus content (38.87 P_2O_5 mg.100 g^{-1}) and a very high potassium content (50.50 K_2O mg.100 g^{-1}). In the 20-40 cm layer, the values are lower, which is due to the biological accumulation of these two elements in the soil. For nitrogen, the values in the 0-20 cm layer are lower than in the 20-40 cm layer, due to the crop uptake and nitrate nitrogen leaching in the depth of the soil profile. The humus content is 10-15% higher than the average for this type of soil. The performed analysis gives us reason that the soil can be used for carrying out research trials.

Micotoxin analysis

Barley, before being sown in a field experiment, was examined for the presence of fumonisins. Samples were screened by 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. In the barley samples was not detected the presence of fumonisin.

Fungicide treatment

The crop was treated with fungicide an active substance of 250 g/l tebuconazole once in the flag leaf formation phase at a dose of 1000 ml.ha^{-1}. Barley variety “Veslets” of the third reproduction was used in the trial.

Experiment scheme

1. Control - healthy seeds whose plants have not been treated with fungicide.
2. Mechanically damaged seeds whose plants have not been treated with fungicide.
3. Healthy seeds whose plants have been treated with a fungicide.
4. Mechanically damaged seeds whose plants have been treated with a fungicide.

Each treatment has two replicates each with an area of 10 m^2. The entire area of the experimental field is 100 m^2.

Harvesting took place on 03/08/2023. Three squared meters were randomly harvested from each replicate. The grain of all six squared meters for each treatment was measured and converted to kg per ha.

Statistical analysis

Statistical processing was done using the Statgraphics 19 software. The statistical procedure performs a one-way analysis of variance for yield.

RESULTS AND DISSCUSSION

The analysis shows the effect of the fungicide used on treated and untreated barley grains, as well as the difference in their yields. The highest yield of 6700 $kg.ha^{-1}$ was found for the health seeds treatment and fungicide treated plants (Table 1). It is follow by the damaged seeds of plants treated with a fungicide. The lowest yield was recorded in the treatment with damaged seeds and plants not treated with fungicide (4850 $kg.ha^{-1}$). In this survey there was also a tendency for treatments with healthy seeds to have a higher yield than treatments with damaged seeds. Apparently, even the smallest deficiency of nutrients in the initial phase of plant development has an adverse effect on the overall development of plants including grain yield.

The results in Table 1 also show various statistics for yield for each healthy and damage seeds treated and untreated with fungicide. Coefficients of variation indicate the quality of the investigations and the results obtained. Since they are below 30% (i.e. 1.16636%–2.95507%), we can consider that the research has very good accuracy and the obtained indicators are basis for credibility.

It should be noted that the used fungicide affects both the yield and the production of mycotoxins. Taking into account that fumonisins are field mycotoxins we performed mycotoxicological analysis of the harvested barley grains for the presence of these mycotoxins. In harvest obtained from plants of healthy and damaged grains treated with fungicide, the presence of fumonisins was not detected. The presence of fumonisins was only found in harvest obtained from plants of healthy seeds not treated with fungicide−0.234 mg/kg and 0.274 mg/kg in grains of plants of mechanically damaged untreated seeds respectively, Table 2.

Besides, it is possible that the favorable climatic conditions also have an influence on the production of the mycotoxins. However, in the absence of phytopathogens on the plants treated with fungicide, we could mentioned of a positive influence of the active substance tebuconazole on the development of the plants.

It is noteworthy that in our study the two treatments whose plants were treated with fungi-

Table 1. Summary statistics for yield

Treatments	*Yield*	*Standard deviation*	*Coeff. of variation*
Damaged	4850.0	50.65685	1.16636%
Damaged with fungicide	6100.0	110.3137	1.85471%
Healthy	5800.0	140.1421	2.4383%
Healthy with fungicide	6700.0	190.799	2.95507%

Table 2. Presence of mycotoxins in grains of plants treated and untreated with fungicide

Mycotoxins	Treatments of grains			
	Healthy	Damaged	Healthy with fungicide	Damaged with fungicide
Fumonisin mg/kg	0.234	0.274	-	-

cide have a higher yield than their counterparts in treatments with plants not treated with fungicide.

Probably the plants that have not been treated with a fungicide contain traces of *Fusarium* spp., whose pathogenic influence is most often expressed in disrupting the metabolism of the plant, and from there it also affects the yield of grain.

In confirmation of the results above Darby and Emick (2022) showed that treatment of barley with fungicides reduced *Fusarium* mycotoxin levels and increased yield. The application may have improved the overall health of the plants. Hrivna (2003) showed similar results where after application of two fungicides she found the barley yield to increase in 10%. Use of fungicides reduces the occurrence of fungal diseases and thereby reduces yield losses, increasing the economic profit (Sooväli and Koppel, 2009).

Yang et al. (2001) also concluded that application of fungicides against a combination of leaf and ear diseases could increase barley yield and reduce yield variation.

In Figure 1 applies a multiple comparison procedure to determine which means are significantly different from which others.

Three homogeneous groups of means were formed, within which there were no statistically significant differences. There is no statistically proven difference between the treatment with healthy seeds and the treatment with damaged seeds treated with fungicide and thus they form one homogeneous group (B). The remaining homogeneous groups identified were the treatment with damaged seeds (A) and the treatment with healthy seeds treated with fungicide (C). The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0 % risk of calling each pair of means significantly different when the actual difference equals 0.

CONCLUSION

1. *Fusarium* spp. and its metabolites are capable of reducing the actual yield by 15-20%, causing economic losses to farmers.

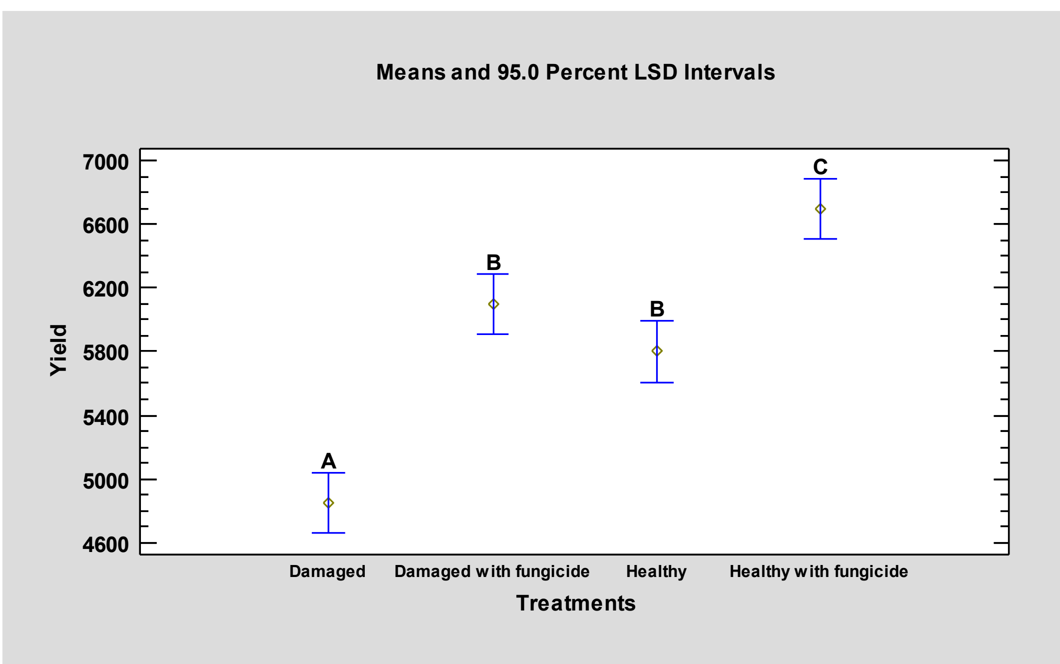

Figure 1. Formed homogenous groups by treatments in relation to yield

2. Even the smallest deficiency of nutrients in the initial phase of plant development adversely affects the overall development of plants, including grain yield.

3. In the treatment with healthy seeds and the treatment with damaged seeds treated with fungicide, the yields are similar, which is statistically proven.

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