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EFFECT OF FUNGICIDES ON DEOXYNIVALENOL CONTENT IN WINTER WHEAT (*Triticum aestivum* L.)

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Abstract

The aim of the present study was to analyze the effect of four fungicidal products on Gibberella ear rot (Fusarium graminearum) on yield quality and quantity of winter wheat (Triticum aestivum L.), cv. Venka 1 and choose the more appropriate application rate. The field experiment was performed in 2022 in Ruse region, Bulgaria. A local variety of winter wheat sensitive to Fusarium ear rot was chosen. The test product (Prothiconazole 174 g/l + Pydiflumetofen 60 g/l) was applied in three dose rates – 0.9, 1.2, and 1,5 l/ha. As reference products, three registered fungicides were selected: Prosaro 420 SC (Prothioconazole 210 g/l + Tebuconazole 210 g/l) - 1 l/ha, Delaro (Prothioconazole 125 g/l + Trifloxystrobin 125 g/l) - 1 l/ha, and Input (Prothioconazole 160 g/l + Spiroxamine 300 g/l) - 1 l/ha and 1.25 l/ha. Nine variants, including untreated inoculated control and seven different treatments, were analyzed. The results showed a significant decrease in the disease severity and incidence after applying all the products and a reduction of deoxynivalenol content in the grain. The yield of all the fungicide treated plots increased.

***Key words**: fungicide, Fusarium graminearum, mycotoxins, winter wheat, yield.*

INTRODUCTION

Wheat is one of the most commonly grown crops worldwide (Khaneghah et al., 2018). The wheat-based products could be categorized as the primary sources of protein and energy of vegetable origin (Jones, 2005). Wheat grains are usually processed into flours which are used for culinary purposes to produce bread, pasta, and cookies or could be used in other industrial sectors. Wheat is highly relevant from both economic and nutritional points of view, but unfortunately, it is susceptible to fungal contamination. The fungal disease may result in the reduction of the grain quality of wheat and the production of mycotoxins (toxic secondary metabolites that are formed by fungi) (Filtenborg et al., 1996).

One of the diseases of great economic importance in wheat is Fusarium head blight (FHB) (Edwards, 2004). The infection causes a reduced yield because of shrunken grains and results in poor milling and malting quality and the contamination of grains with mycotoxins. The most important pathogens of wheat causing FHB are *Fusarium graminearum* and *F. culmorum*. They are more pathogenic than other species and they produce higher amounts of mycotoxins, especially deoxynivalenol (DON). During late spring, the conidia are splash dispersed via rainfall. The dry weather usually promotes sporulation (Edwards, 2004). Mycotoxins are a large group of substances with a small molecular weight with diverse structures. The main producer of these compounds is the secondary metabolism of some filamentous fungi or molds. They are able, under suitable temperature and humidity conditions, to develop on various foods and feeds and to cause serious risks to human and animal health (Zain, 2010). Deoxynivalenol (DON) is a mycotoxin that contaminates grains, cereal-based food, and feed. It belongs to the group of trichothecenes and engenders intense gastrointestinal adverse effects like vomiting (emesis) in animals and humans. The long-term dietary exposure to DON in animals leads to weight gain suppression, altered nutritional efficiency, and anorexia (EFSA 2013). Sensitivity to DON depends on the specific metabolism, the absorption, circulation, and elimination of DON by the organism (Sobrova et al., 2010). It is observed that some ruminants such as cattle, sheep, goats, and deer are less sensitive to the negative effects of mycotoxins than the non-ruminant organisms. However, if the animals consume mycotoxin-contaminated feed for a long period, this could alter the growth, reproduction, and quality of production (milk, beef, or wool) (Hussein and Brasel, 2001). Therefore, according to Morgavi & Riley (2007), DON is the primary compound, which is responsible for economic losses due to the reduction of performance in animal husbandry. The consumption of DON-contaminated wheat and wheat-based products, such as bread and pasta, could also endanger human health (Khaneghah et al., 2018). The content of mycotoxins in plants can be influenced using different agro technical practices, the most successful of which is the use of fungicides. Fusarium head blight infection occurs at flowering and numerous studies have shown that fungicide application is most effective when applied from early to mid-flowering (Edwards, 2022).

MATERIALS AND METHODS

Experimental design

The field experiment was set up in Ruse Region, Bulgaria in 2022. *Triticum aestivum* L. cv. Venka 1 was selected as a test object. The experiment was set up using the block plot method. Each plot was with the size of 24 m^2. The plants were sown on 27 October 2021 with a row spacing of 3 cm and spacing within row of 10 cm. The sowing rate was 250 kg/ha of seeds. The date of emergence was 18 November 2021. The yield was harvested on 12 July 2022.

Artificial inoculation

The test plots were artificially contaminated with *Fusarium graminearum*. Inoculation of the disease was conducted using a spraying spore suspension method. Conidia solution was prepared at concentration of 10^5 to 10^6 conidia/ml to use at a final spray concentration of 10^3 to 10^4 conidia/ml. The inoculum was applied one day before the application of the test products, at BBCH stage 63 (flowering), as flowering is the most sensitive growing stage for artificial inoculation.

Application data

The test plots include inoculated untreated control and eight different treatments. The control plants were not treated with a fungicidal product. The test product contains Prothioconazole 174 g/l + Pydiflumetofen 60 g/l, and it was applied in three dose rates to select the most appropriate application dose. As reference products, three already registered fungicides were used. These were Prosaro 420 SC (applied in one dose), Delaro (applied in two doses), and Input (Bayer Crop Sciences), applied also in two dose rates. Prosaro 420 SC contains Prothioconazole 210 g/l + Tebuconazole 210 g/l, Delaro contains Prothioconazole 125 g/l + Trifloxystrobin 125 g/l. Input contains 160 g/l Prothioconazole + 300 g/l Spiroxamine. The list of the experimental variants included nine variants and is given in Table 1. All the products were applied one day after the artificial inoculation in BBCH 63 (flowering). The spray volume was 500l/ha.

Table 1. List of treatments

Variant	Description
1	Untreated control
2	Test Product 0.9 l/ha
3	Test Product 1.2 l/ha
4	Test Product 1.5 l/ha
5	Prosaro 420 SC 1 l/ha
6	Delaro 1 l/ha
7	Delaro 1.25 l/ha
8	Input 1 l/ha
9	Input 1.25 l/ha

Mycotoxin content estimation

The mycotoxin concentration was estimated using high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) meeting VLM 92 2010.

Statistical analyses

The data are presented as mean of 4 replicates. The experimental results were statistically processed with the SPSS program using a one-way ANOVA dispersion analysis and Duncan's comparative method, with the validity of the differences determined at a 95% significance level. The different letters (a, b, c, d) after the mean value show statistically significant differences between the variants.

RESULTS AND DISCUSSIONS

Data about the *Fusarium graminearum* severity is presented in Figure 1. It is seen that the highest disease severity is observed in variant 1 - the untreated control (about 13.7%). On all of the test plots, which were treated with pesticides, there was a great reduction in the disease severity compared to the control. In variant 2 and variant 6, disease severity was about 2.1 and respectively 2.3%, and in variant 3 - less than 2%. In variants 4, 5, 7, 8, and 9, the percentage of the infected area was lower that 1%. The results show that the application of the test product is able to reduce significantly the disease severity in comparison to the untreated plots. The best result is obtained using the higher rate of application of 1.5 l/ha. The lowest disease severity is observed on variant 9, where Input in the dose of 1.25 l/ha was applied.

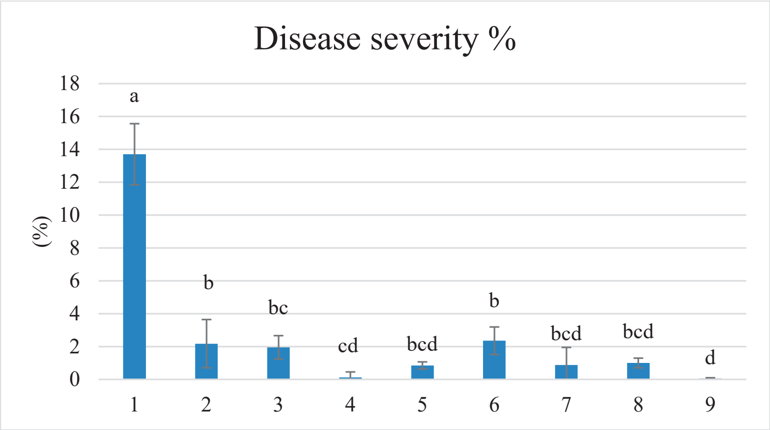

Figure 1. Disease severity (% area) of *Fusarium graminearum* in winter wheat after artificial contamination and pesticide application

The disease incidence measurement shows that the highest number of infected plants was observed on the control plots (Figure 2). Almost 45% of the control plants had disease symptoms. The number of the infected plants treated with the test product was significantly lower. Only 12% in variant 2 and about 10.5% in variant 3, respectively. The lowest rate of disease incidence was measured on the plots treated with 1.5 l of the test product (variant 4) and the plants treated with Input 1.25 l/ha (variant 9). On the other experimental plots, the disease severity varied between 9 and 4%.

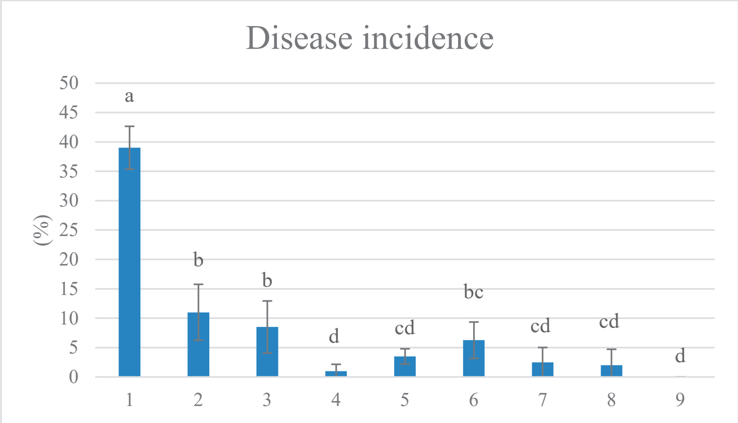

Figure 2. Disease incidence (%) of *Fusarium graminearum* in winter wheat after artificial contamination and pesticide application

It was expected that if the disease severity and incidence were so significantly reduced after the application of the fungicidal products, the content of mycotoxins would be reduced too. The analyses of the mycotoxin content are presented in Figure 3. All the variants, which were treated with a fungicide, showed a significantly lower mycotoxin content. The content of deoxynivalenol is presented in Figure 3.

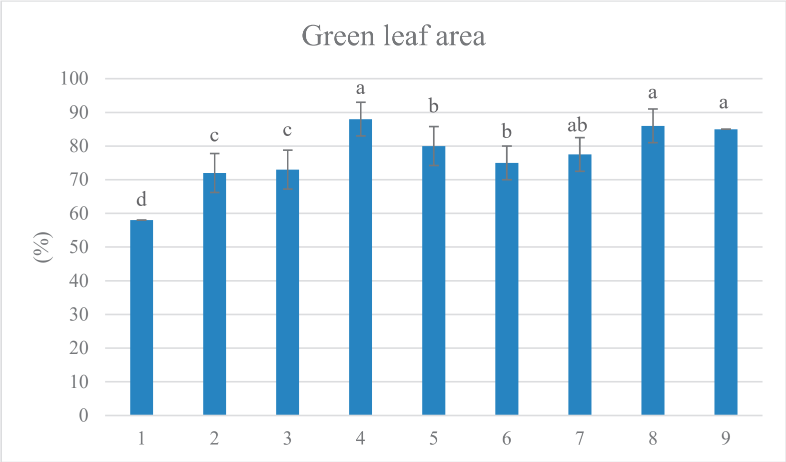

Figure 3. Green leaf area % in winter wheat after the contamination with *Fusarium graminearum* and pesticide application

According to Pepler et al. (2005), the use of fungicides is associated with yield enhancement, and the reason for that is the improvement of photosynthesis during grain filling. The physiological status of the green leaf area is maintained by restricting the development of known pathogens (Ruske et al., 2003) or even unknown pathogens which are hardly identified in the field (Bertelsen et al., 2001). Sometimes the positive effect of the pesticide application is due to the direct physiological effects of the fungicide itself on the plant (Grossmann and Retzlaff, 1997). The percent of the green leaf area, which had no visible symptoms of the disease, is presented in Figure 3. The lowest percent of green area is measured in the untreated control and in variant 9. The highest percent is measured in variant 4 and variant 8. According to Morgavi & Riley (2007), mycotoxins, especially DON, are characterized as a major cause of economic loss due to reduced performance, although they are not considered acutely toxic to farm animals. In concern to human safety, The Scientific Committee on Food (SCF) established a temporary Tolerable Daily Intake (TDI) of 1 μg/kg body weight (b.w.) per day. This was established after a 2-year feeding study in mice with decreased gain body weight, based on a No Observed Adverse Effect Level (NOAEL) of 100 μg/kg b.w. per day (SCF, 2002). In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) proposed an Acute Reference Dose (ARfD) at 8 μg/kg body weight. According to EFSA (2013), high consumers and young children were exposed to DON at levels close to or even higher than the TDI (EFSA, 2013).

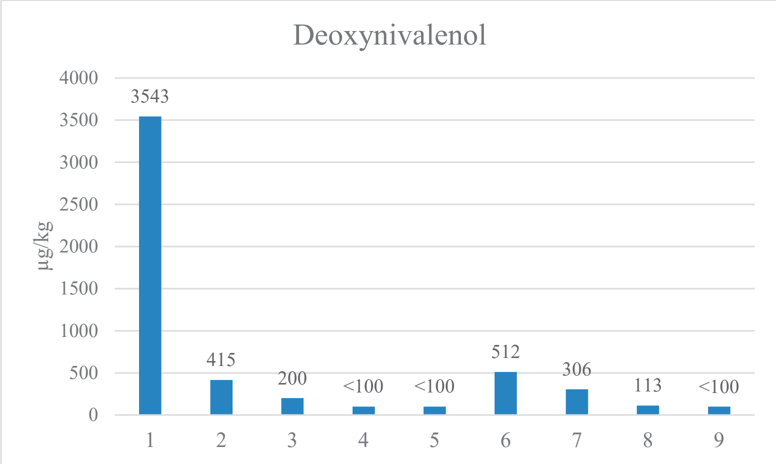

Figure 4. Content of deoxynivalenol (µg/kg) of winter wheat after the contamination with *Fusarium graminearum* and pesticide application

In this regard, the minimum DON content measured in the current experiment was 100 µg/kg. The content of DON is presented in Figure 4. It is seen that the lowest amounts of mycotoxins are observed in variants 4, 8, and 9, where the content of deoxynivalenol is so small that it could not be measured (as the methodology of the accredited laboratory detects concentrations of a minimum of 100 µg/kg). The pesticide application reduced the amounts of the toxic secondary metabolite to very low levels. The rate of reduction depends on the product and its application rate. It is observed that the content of mycotoxins is reduced between 7 and 50 times compared to the control. As expected, the lowest disease severity and incidence resulted in a better yield. The results of the yield of winter wheat are presented in Figure 5. Compared to the untreated control, the pesticide application yielded an increase in all the test variants where fungicides were applied. The highest yields are measured in variant 4 and variant 8, where the test product in a dose of 1.5 l/ha and Input in a dose of 1 l/ha, respectively, were used.

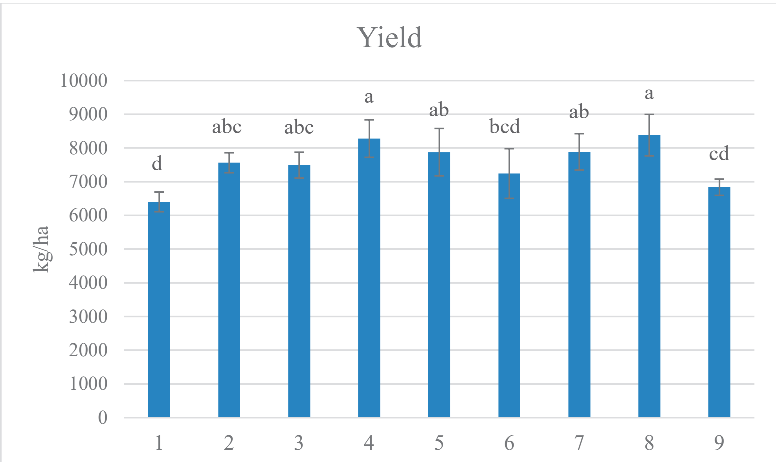

Figure 5. Yield of winter wheat after the contamination with *Fusarium graminearum* and pesticide application

There are few scientific works on the efficacy of pydiflumetofen (succinate dehydrogenase inhibitor) against FHB. They observe either its effect as a solo product or as a co-formulation with propiconazole (Singh et al., 2020; Xia et al., 2021). According to Edwards & Godley (2010) the pesticide Proline containing propiconazole was able to reduce the infection of head blight on oats and the content of DON, respectively. In the research of Edwards (2022) it is pointed out that the new fungicide, containing pydiflumetofen in a co-formulation with prothioconazole (triazole), was significantly better at reducing FHB disease and resulted in a reduction of DON contamination of grain than either pydiflumetofen or prothioconazole alone.

CONCLUSIONS

The current experiment aimed at exploring the effect of different pesticides on yield and the content of mycotoxins, namely DON, in winter after the artificial inoculation with *Fusarium graminearum*. The results show that all the tested fungicides could reduce the disease severity and incidence of *Fusarium graminearum* on winter wheat. As a result, the content of mycotoxins in grain was significantly lower in all the test variants compared to the untreated control. This led to an increase in the yield in the plots where fungicides were applied. The highest yield was observed in variant 4 after applying the test product containing Prothioconazole 174 g/l + Pydiflumetofen 60 g/l in a dose of 1.5 l/ha and in variant 8, where Input was applied in a dose of 1 l/ha. The strongest reduction of the mycotoxin content was measured in variant 4, variant 8, and variant 9, where the test product in a dose of 1.5 l/ha, Input in a dose of 1 l/ha, and a dose of 1.25 l/ha was applied.

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