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RESEARCHES ON THE IMPACT OF FERTILIZATION ON FUSARIOTOXINE (DEOXYNIVALENOL, DON) PRODUCTION IN WHEAT GRAINS

Roxana ANDRONE, Mihai GIDEA, Ioan ROSCA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: ioanrosca_usamv@yahoo.com

Abstract

Fusarium is a major disease in most wheat growing areas around the world, including Romania. Five species of the genus Fusarium can cause this to wheat, Gibberella zeae Schwain (Petch.) (with anamorphous Fusarium graminearum Schwabe) is the predominant pathogen in many parts of the world. This disease not only causes crop losses, but also contamination of wheat with fusariotoxine (especially deoxynivalenol, DON) which, due to cytotoxic and immunosuppressive properties, are harmful to humans and animals. DON content was analyzed in samples of wheat harvest 2012 and 2013, taken from field plots (mixture from 5 repetition for each variant) The maximum Fusariotoxine limit allowable for cereals, regulated by Commission Regulation (EC) No. 1126/2007 of 28 September 2007 (amending Regulation (EC) No. 1881/2006), is 1.25 mg / kg (ppm). The protection of crops against mycotoxin contamination using fungicides is not always productive; the application of bio stimulant seems to be promising in this respect. At some specific stages of wheat (Triticum aestivum L.) growth, commercially available organic growth stimulants (bio stimulants) were tested from 2012 and 2013 in experimental field. The purpose of our work was to evaluate the effect of bio stimulants treatment on mycotoxin content in grain harvest. The application of bio stimulants in recommended dosages, depending of the year (connected with climatic conditions) and variant, has been found that could reduce the content of DON till 0.5405 and 0.781 mg/kg (ppm), in 2012 and 2013 respectively.

Key words*: bio stimulant, wheat, evolution of deoxynivalenol (DON).*

INTRODUCTION

Fusarium head blight (FHB), also known as scab, is a fungal disease of small grain cereals that has become of increasing international importance in recent years. FHB is caused by several species of the fungal pathogen, Fusarium. The most important species is *Fusarium graminearum*, since it can result in the most yield loss and is responsible for the production of toxin in the grain (http://www.agriculture.gov.sk.ca/fusarium-head-blight). Fusarium is one of the most widespread and damaging diseases in cereals in favorable areas causing losses of 10-20% of production. Although epidemics of Fusarium in wheat, in Romania, are not common, the disease is important, during a certain period of time, attack recorded in five from nine localities, but in one third of the locations, the incidence was negligible, although the disease is considered as the most important wheat disease in Romania. The disease occurs with greater intensity in Romania representing a particular problem in the production of healthy seed (Saulescu, 1993). Outbreaks of this disease were recorded in 1970 and 1975, many areas of common wheat crop attack rises to 65-80%, followed by a strong shriveled grains. The disease was recorded most frequently under hot, wet climatic conditions where significant yield losses and mycotoxin accumulation in grain were reported (Parry et al., 1995). In the UK, warming is considered as the main factor involved in frequency increasing of fusarium diseases (Gosman et al., 2007). Mycotoxins are toxic, secondary metabolites produced during the fungal infection process. Deoxynivalenol or DON is the most common mycotoxin associated with FHB and is produced in high amounts by *F. graminearum*. Compared to other mycotoxins, DON is one of the least toxic but can still result in reduced feed consumption or feed refusal, especially when fed to non-ruminants (http://www.agriculture.gov.sk.ca/fusarium-head-blight). There is some evidence that FHB can be affected by fertilizer regimes. Martin et

al. (1991) and Lemmens et al. (2004) observed that increasing the amount of nitrogen applied to cereals resulted in increased incidence of FHB or *Fusarium*-infected grain, however Lori et al. (Lori et al., 2009) reported that favourable weather conditions are a more important factor for FHB infection than tillage practice and fertilizer treatments. Plant biostimulants are diverse substances and microorganisms used to enhance plant growth. The global market for biostimulants is projected to increase 12% per year and reach over $2,200 million by 2018. Despite the growing use of biostimulants in agriculture, many in the scientific community consider biostimulants to be lacking peer-reviewed scientific evaluation (Calvo et al., 2014; Anonymous, 2013). According to the same study, the largest market for biostimulants in 2012 was Europe. The European biostimulants industry council (EBIC) reported that in 2012 over 6.2 million hectares were treated with biostimulants in Europe (defined as the European Economic Area) (European Biostimulants Industry Council, 2013).
High fertilizer rates significantly increased spring wheat grain infection with *Fusarium* spp. tillage systems had no significant influence on *Fusarium* infection level; however, they had indirect effect on mycotoxin content in separate years (Suproniené et al., 2012).

MATERIALS AND METHODS

Experimental trials were done at Moara Domneasca (experimental farm belonging to University, near Bucharest), variants and moment of treatments are presented in table 1. In 2012 were 3 experimental variants, in 5 replicates, plot area 13 x 8=104 m^2. Sowing data October 25, 2011 after sunflower (hybrid Expres), cultivar: Dropia, sowing rate 250 kg/ha. Basic fertilization (N60/P60), before sowing under ploughing; herbicide applying on May 10 with Granstar (tribenuron methyl), 40g/ha. In 2013 were 4 experimental variants in 4 replicates, plot area 13.5 x 7.5 = 101.25 m^2). Sowing data October 17, 2012 after sunflower (hybrid Favorit), cultivar Dropia, sowing rate 250 kg/ha, basic fertilization (N60/P60), before sowing under ploughing. At growth stage BBCH 31-33 was applied herbicide Pelican Delta 606 WG (diflufenican 60% + metsulfuron metil 6%), at growth stage BBCH 61 was applied fungicide Acanto Plus (picoxistrobin 200 g/l + ciproconazol 80 g/l).

Table 1. Variants and moment of treatments

Year	Product	Rate (l/ha)	No. applications	Growth Stage
2012	**Megafol**	2 + 2	1 1	BBCH 31-33 BBCH 61
	Megafol	3	1	BBCH 31-33
	Megafol	3	1	BBCH 61
	Check	-	-	-
2013	**Megafol**	2	1	BBCH 31-33
	Megafol	3	1	BBCH 61
	Cropmax	2	1	BBCH 31-33
	Cropmax	3	1	BBCH 61
	Check	-	-	-

DON content was analyzed in samples of wheat harvest 2012 and 2013, taken from field plots (mixture from repetitions for each variant). It was used a kit DON Fast Ridascreen from R-Biopharm (Figure 1) and read absorbance reader Stat Fax 2100 (Figure 2). DON concentration in mg/kg corresponding to each sample absorbance it was red using the calibration curve. To express qantitative immunoenzymatic reaction of Ridascreen kit it was used special software, RIDA® SOFT Win.

Figure 1. Reagent kit Ridascreen fast DON

Figure 2. Read absorbance reader Stat Fax 2100

RESULTS AND DISCUSSIONS

Fusarium disease not only causes crop losses, but also contamination of wheat with fusariotoxine (especially deoxynivalenol, DON) which, due to cytotoxic and immunosuppressive properties, are harmful to humans and animals. DON content was analyzed in samples of wheat harvest 2012 and 2013, taken from field plots (mixture from 5 repetition for each variant). The maximum Fusariotoxine limit allowable for cereals, regulated by Commission Regulation (EC) No. 1126/2007 of 28 September 2007 (amending Regulation (EC) No. 1881/2006), is 1.25 mg/kg (ppm). In 2012 taking into consideration that the maximum Fusariotoxine limit allowable for cereals, regulated by Commission Regulation (EC), is 1.25 mg / kg (ppm), so only at check is exceeded this limit 1.963 (ppm). Less DON content was registered at variant Megafol, 2 applications at rate l/ha, the variant has a DON content (0.5405 mg/kg (ppm). The variant Megafol, 1 application at rate l/ha: 3 l/ha (at growth stage BBCH 31-33), has a DON content [0.9765 mg/kg (ppm)], close to maximum Fusariotoxine limit allowable for cereals [1.25 mg/kg (ppm)]. The variant Megafol, 1 application at rate l/ha: 3 l/ha (at growth stage BBCH 61), has a DON content [1.2 mg/kg (ppm)], close to maximum Fusariotoxine limit allowable for cereals [1.25 mg/kg (ppm)]. In 2013 as in previous year, only at check is exceeded this limit 1.942 (ppm). Less DON content was registered at variant Megafol, applications at rate 2 l/ha at Growth Stage BBCH 31-33 [0.519 mg/kg (ppm)], also under the level of Cropmax applications at rate 2 l/ha at Growth Stage BBCH 31-33 [0.781 mg/kg (ppm)]. The variant Megafol, application at rate 3 l/ha (at growth stage BBCH 61), has a DON content [0.99 mg/kg (ppm)], under to maximum Fusariotoxine limit allowable for cereals (1.25 mg/kg (ppm), also under the level of Cropmax 3 l/ha (at growth stage BBCH 61), has a DON content [1.21 mg/kg (ppm)].

Table 2. Variants and moment of treatments

Year	Product	Rate (l/ha)	No. applications	DON content ppm (mg/kg)
2012	**Megafol**	2 + 2	1 1	**0.5405**
	Megafol	3	1	**0.9765**
	Megafol	3	1	**1.2**
	Check	-	-	***1.963***
2013	**Megafol**	2	1	**0.519**
	Megafol	3	1	**0.99**
	Cropmax	2	1	**0.781**
	Cropmax	3	1	**1.21**
	Check	-	-	***1.942***

CONCLUSIONS

- Our experiment prove that application of biostimulants reduce quantity of DON under The maximum Fusariotoxine limit allowable for cereals, regulated by Commission Regulation (EC) No. 1126/2007 of 28 September 2007 (amending Regulation (EC) No. 1881/2006), is 1.25 mg / kg (ppm).
- In 2012 as in 2013, only at check is exceeded this limit [1.963, in 2012 and 1.942, in 2013 mg/kg (ppm)].
- Less DON content was registered at variants, applications at rate 2 l/ha at Growth Stage BBCH 31-33 than at the variants, application at rate 3 l/ha (at growth stage BBCH 61).

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