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Mycotoxin Level in Winter Wheat Grain as Impacted by Nitrogen and Manganese Fertilisation

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Abstract: A field experiment with winter wheat (*Triticum aestivum* L.) cultivation was conducted at the Research and Education Centre in Tomaszkowo, Poland (53°72′ N; 20°42′ E) in the years 2013–2016. Fertilisation with nitrogen at 150 and 200 kg ha^{-1} and foliar application of manganese at 0.5 and 1.5 kg ha^{-1} were the research factors. Wheat infestation by *Fusarium* spp. was determined by the habitat conditions during crop growth. Neither nitrogen nor manganese fertilisation affected the presence of *Fusarium* spp. symptoms on wheat ears, but the infestation intensity decreased with increasing nitrogen and manganese content in the grain. Only the level of deoxynivalenol (DON) was correlated with *Fusarium* spp. infestation. Increasing the nitrogen fertilisation rate from 150 kg ha^{-1} to 200 kg ha^{-1} resulted in higher grain contamination with toxins. Supplementation of nitrogen fertilisation with manganese reduced the number of mycotoxins in wheat grain. The grain yield was mainly affected by the varied weather conditions during the wheat-growing periods. Neither nitrogen nor manganese fertilisation differentiated the wheat grain yield. The objective of this study was to examine the impact of the weather conditions and nitrogen and manganese fertilisation on the grain yield, occurrence of *Fusarium* head blight and mycotoxin level in winter wheat grain.

Keywords: nitrogen; foliar fertilisation with manganese; mycotoxins; DON; NIV; ZEA; MON

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1. Introduction

Of all the agrotechnical procedures performed in intensive wheat cultivation, fertilisation—which includes the application of both micro- and macronutrients—has the strongest impact on yield, its chemical composition and, as a consequence, its quality [1]. When not properly balanced, fertilisation can impair the soil's physicochemical characteristics and fertility [2].

Macro and micronutrient fertilisation is of great importance in boosting wheat resistance to infestation with pathogens that cause root, culm base, leaf and ear diseases [3]. Cereal fungal diseases decrease grain yield and impair its quality [4]. The loss caused by reducing the grain mass and the number of grains per ear can reach 10 to 40% [5]. Strong plant infestation reduces the assimilation area and photosynthesis intensity [6].

Cereals are susceptible to diseases caused by *Fusarium* spp. fungi, and wheat is particularly susceptible to *Fusarium* head blight [7]. Ear infestation by *Fusarium* spp. can cause a loss in yield of up to 15–20%, and even 60% in extreme cases, with infested grain being of low quality [8]. The intensity of *Fusarium* head blight depends largely on weather conditions [9]. According to Xu et al. [10], environmental conditions influence the processes of infection and colonisation in various ways, as well as an abundance of

species of the genus Fusarium spp. Humidity determines the intensity of the disease, while precipitation determines inoculum levels [11]. Significant infestation of spikes by *Fusarium* spp., including *F. graminearum*, *F. culmorum*, *F. poae*, and *F. avenaceum*, requires substantial moisture persisting for at least 24 h and temperatures exceeding 15 °C [12]. Factors influencing wheat infection with *Fusarium* spp. may include the following: agricultural technology, forecrop, fertilisation and use of fungicides [8,13]. Hazard of *Fusarium* spp. occurrence in wheat also depends on the genetically determined variety resistance [14]. According to Spanic [15], infected seeds with *Fusarium* spp. were smaller with lower 1000 kernel weight and had less endosperm, which resulted in increased protein content.

Grain infestation with fungi of *Fusarium* spp. results in the formation of secondary metabolites, such as mycotoxins. In Poland, these include mainly trichothecenes A and B (deoxynivalenol (DON), nivalenol and T2/HT-2 toxins), fumonisins and zearalenone (ZEA) [16]. Mycotoxins are significant cereal contaminants, generating economic losses in the fodder and food industry and having an adverse impact on human and animal health [17]. The presence and concentration of mycotoxins produced by fungi of *Fusarium* spp. in wheat depend on multiple factors, e.g., toxigenic potential of a specific fungus species [18], weather conditions [4,19], presence of competitive microflora [20], tolerance of the cultivar [21] and growth conditions [22]. The literature reports on the nitrogen impact on *Fusarium* spp. occurrence and mycotoxin production are inconclusive. According to Aufhammera et al. [23], Krnjaja et al. [24] and Cwalina-Ambroziak et al. [25], infestation by *Fusarium* spp. and mycotoxin production was not related to the amount of nitrogen supplied. A study conducted by Supronienė et al. [26], and Podolska et al. [27] indicates a distinct impact of N fertilisation on *Fusarium* spp. occurrence and mycotoxin production. Bernhoft et al. [28] and Champeil et al. [29] found nitrogen fertilisation to have the potential to damage cellular wall structures in plants and adversely impact plant chemical composition, thereby making plants more susceptible to pathogen invasions. Nitrogen excess makes plants more luxuriant and the stand denser, resulting in higher moisture content, which favours infections by *Fusarium* and mycotoxin production [29].

Micronutrients are involved in physiological and biochemical mechanisms of defence against pathogens. Manganese participates in the synthesis of lignin, which constitutes a physical barrier against pathogen infiltration [30,31].

There have been multiple studies on the impact of nitrogen fertilisation on *Fusarium* blight and mycotoxin occurrence in cereals, but there have been none dealing with a combined impact of nitrogen and manganese fertilisation and habitat conditions on their presence in wheat.

2. Materials and Methods

2.1. Experimental Conditions

The field experiment with winter wheat (*Triticum aestivum* L.) cultivation was conducted at the Research and Education Centre in Tomaszkowo, Poland (53°72′ N; 20°42′ E) in the years 2013–2016. The experiment was set up in the randomised block design in triplicate. A description of the agrotechnical procedures, the soil characteristics before the experiment was set up and the winter wheat main growth stages are shown in Table 1.

Table 1. Description of the agrotechnical procedures, soil characteristics and main growth stages of winter wheat.

Item	Description
Land tilling (2013–2016)	Postharvest tilling (August)—disking (10 cm). Pre-sowing tilling (September)—ploughing (20 cm), harrowing, fertiliser-covering harrowing.
Cultivar description	Smuga: a quality cultivar, very early ear formation, a large mass of thousand grains, medium resistance to diseases (*Fusarium* head blight 7.2 on the scale of 1–9°).
Sowing description	Area of plots for sowing 2.2 m × 4.5 m = 9.9 m^2 Plot row drill, sowing with rows spaced every 12 cm, sowing density 550 grains m^{-2}

Table 1. *Cont.*

Item	Description
Fertilisation *	The same on each plot: phosphorus: 79.6 kg N ha^{-1} (triple superphosphate, 20.1% P), potassium: 83.1 N ha^{-1} (potassium salt, 49.8% K).
Protection against weeds (monocotyledonous and dicotyledonous)	Spraying against monocotyledonous weeds: Axial 100 EC 0.4 L ha^{-1} (pinoxaden—50 g L) Spraying against dicotyledonous weeds: Mustang Forte 195 SE 1.0 L ha^{-1} (florasulam 5 g L, aminopyralid 10 g L, 2.4 D 180 g L)
Protection against diseases	Not applied
Protection against pests	Not applied
Harvesting	Area of plots for harvesting: 2.0 m × 4.0 m = 8.0 m^{-2} Plot combine harvester (Wintersteiger Classic 1540, STEURER Trocknungs- und Aufbewahrungssysteme GmbH, Altach, Austria)
Main growth stages	Sowing dates (BBCH 00): 11.09.2013, 15.09.2014, 18.09.2015 Emergence (BBCH 09): 18.09.2013, 24.09.2014, 25.09.2015 Beginning of tillering (BBCH 21): 21.10.2013, 20.10.2014, 22.10.2015 Beginning of heading (BBCH 51): 26.05.2014, 26.05.2015, 25.05.2016 Harvest date (BBCH 91): 25.07.2014, 03.08.2015, 25.07.2016
Soil characteristics	Grey-brown podzolic soil, with the granulometric composition of medium silty clay. According to the World Reference Base for Soil Resources (WRB, 2014), this corresponds to a soil profile called Haplic Cambisol. Slightly acidic (in KCl solution with pH 5.7); the content of $C_{organic}$: 10.1–10.5 g kg^{-1}; Nt_{otal}: 0.97–1.02 g kg^{-1}; P: 83.3–86.0 mg kg^{-1}; K: 145.0–155.1 mg kg^{-1}; Mg: 68.7–72.3 mg kg^{-1}; and Mn: 145.0–150.2 mg kg^{-1}

* N and Mn fertilisation in Table 2.

2.2. Design of Experiment

Fertilisation with nitrogen at 150 and 200 kg ha^{-1} and foliar application of manganese at 0.5 and 1.5 kg ha^{-1} were the research factors. The nitrogen and manganese rates and the dates of application are given in Table 2.

Table 2. Design of the field experiment. Dose and date of application fertilisers used in the field experiment.

Treatment	Mineral Component/Development Phases/BBCH Phase/Fertiliser Form/Dose				
	N				Mn
	Pre-Sowing	Tillering/BBCH 25–29	Stem Elongation/BBCH 30–31	Heading/ BBCH 51–52	Stem Elongation/ BBCH 30–31
	Urea 46% ($CO(NH_2)_2$)	Ammonium Nitrate 34% (NH_4NO_3)	Ammonium Nitrate 34% (NH_4NO_3)	Urea 46% ($CO(NH_2)_2$), Foliar Application of a 10% Solution	0.5% Solution of $MnSO_4$ $5H_2O$
Without fertilisation	-	-	-	-	-
150 kg N ha^{-1}	40	70	30	10	-
150 kg N ha^{-1} + 0.5 kg Mn ha^{-1}					0.5
150 kg N ha^{-1} + 1.5 kg Mn ha^{-1}					1.5
200 kg N ha^{-1}	40	80	60	20	-
200 kg N ha^{-1} + 0.5 kg Mn ha^{-1}					0.5
200 kg N ha^{-1} + 1.5 kg Mn ha^{-1}					1.5

2.3. Weather Conditions

The weather conditions in the years of study varied (Table 3). The average temperatures between September and August were higher by 0.6 °C than the multi-year average

during all three years of study. September 2013 was the coldest month during the period of initial plant growth (lower temperature by 2.3 °C than in 2014 and 2015). A lower temperature in September 2013 caused better tillering, and a higher temperature in November extended their growing period. The rapid temperature decrease in early January 2016 caused considerable plant freezing, which subsequently affected the yield. The temperatures in winter helped plants to last through the cold period during the other years of the study. Higher temperatures in spring (March–April) 2014 induced the rapid emergence of wheat.

Table 3. Monthly air temperature and monthly rainfall in the 2013–2016 season. Meteorological data against the years 1981–2010 (data obtained from the Meteorological Station at Tomaszkowo, (53°71′ N, 20°43′ E), Poland.

Growing Season	Month												
	IX	**X**	**XI**	**XII**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**Av. IX–VIII**
	Temperature												
2013/2014	11.3	8.9	5.0	2.3	−4.0	1.2	5.1	8.8	13.0	14.4	20.4	17.1	8.6
2014/2015	13.6	8.7	3.7	−0.4	0.4	0.5	4.2	6.7	11.8	15.5	17.5	19.8	8.5
2015/2016	13.5	6.1	4.8	3.4	−4.0	2.3	3.0	7.4	13.7	17.1	18.1	17.1	8.5
1981–2010	12.8	8.0	2.9	−0.9	−2.4	−1.7	1.8	7.7	13.5	16.1	18.7	17.9	7.9
	Rainfall												
	IX	X	XI	XII	I	II	III	IV	V	VI	VIII	VIII	Sum IX–VIII
2013/2014	101.1	16.0	18.0	27.7	48.4	8.1	57.7	26.0	32.7	50.8	37.3	86.1	509.9
2014/2015	25.9	15.1	34.0	61.8	46.8	6.8	45.1	38.2	29.7	29.5	81.9	14.3	429.1
2015/2016	63.8	19.4	84.5	56.6	24.7	57.1	21.6	28.8	56.9	69.3	130.4	70.4	683.5
1981–2010	56.9	42.6	44.8	38.2	36.4	24.2	32.9	33.3	58.5	80.4	74.2	59.4	581.7

The highest total rainfall was determined in the growing season of 2015/2016. However, over 20% of the precipitation occurred during the pre-harvest period, hindering the harvest and, in effect, increasing the loss in yield due to freezing. September 2013 was the most humid month during the initial plant-growing period, which had an impact on water accumulation and its effective use during the plant tillering. It is of key importance for achieving better yield to supply sufficient amounts of water during the period of plant emergence (March). That was the case in 2014 and in 2015.

2.4. Determination of N and Mn Content of Grain

Ground grain samples were mineralised in concentrated H_2SO_4 with K_2SO_4 and $CuSO_4$ (as an oxidiser). Protein content in wheat grain was determined by the Kjeldahl method (N × 5.7) in the KjelFlex K-360 apparatus (Buchi, Flawil, Switzerland). For Mn determination, ground grain was mineralised in a mixture of HNO_3 and $HClO_4$ (at 4:1). Manganese content was determined by atomic absorption spectroscopy (AAS) in a Hitachi Z-8200 apparatus (Hitachi, Ltd., Tokyo, Japan).

2.5. The Severity of Fusarium Head Blight (Fusarium spp.) in Winter Wheat

During the wheat-growing period, the intensity of *Fusarium* head blight symptoms was assessed visually in principal growth stage 7: development of fruit—late milk (BBCH 77). An analysis was performed for 25 randomly selected plants on a plot using a 5-degree scale. Following the conversion, the results were given in % as the infestation index. The results for the disease intensity were calculated from McKinney's formula [32] and presented as infestation index in percent:

$$Ii = \frac{\sum(a \times b) \times 100\%}{N \times I}$$

where $\sum (a \times b)$ is the sum of products from multiplying the number of plants (a) on a given degree of scale (b), N is number of plants under analysis and I is the highest degree of the scale.

2.6. Mycotoxin Analysis

All mycotoxins (MON, ZEA, DON, NIV) from plant material were extracted and purified according to the detailed procedure described elsewhere [33,34]. A total of 10 g of ground samples of winter wheat (each sample in three repetitions) were homogenised for 3 min in 30 mL of acetonitrile:water (80:20, v/v) and filtered through Whatman no. 4 filter paper (Whatman International Ltd., Maidstone, UK). The elute was evaporated to dryness at 40 °C under a stream of nitrogen. The dry residue was stored at −20 °C until HPLC analyses. The chromatographic system consisted of a Waters 2695 high-performance liquid chromatograph (Waters, Milford, CT, USA) with the following detectors: a Waters 2996 Photodiode Array Detector with a Nova Pak C-18 column (300 × 3.9 mm) for DON, NIV (λmax = 224 nm) and MON (λmax = 229 nm) analysis; a Waters 2475 Multi λ Fluorescence Detector (λex = 274 nm, λem = 440 nm) and a Waters 2996 Photodiode Array Detector with a Nova Pak C-18 column (150 × 3.9 mm) for ZEA analysis. The mycotoxins were quantified by measuring peak areas and retention times using the calibration curve. The limits of detection were as follows: 1 ng g^{-1} for ZEA; 10 ng g^{-1} for DON, NIV and MON.

2.7. Grain Yield

A plot combine harvester (Wintersteiger Classic 1540, STEURER Trocknungs- und Aufbewahrungssysteme GmbH, Altach, Austria) was used for wheat grain harvest during the technical ripeness stage (BBCH 91). The grain harvested from the plots was used to determine the yield per 1 ha and for further analysis. A hygrometer (GMS v2, Dramiński, Gietrzwałd, Poland) was used to determine the grain moisture content, and the wheat grain yield was determined per 1 ha after converting to the same moisture content of 15% with the scales (WTC 2000, Radwag, Radom, Poland).

2.8. Statistical Analysis

Statistica v.13.1 software was used for the statistical analysis of the study results. Statistical inference consisted of the demonstration of significant differences in fertilisation variants by one-way analysis of variance. Homogeneous groups were determined by Tukey's test. The calculations were made at the level of significance of $\alpha = 0.05$. The Pearson correlation coefficient (r) was calculated, followed by the linear regression equation. The relationships between the two characteristics are shown in the dispersion diagrams.

3. Results

3.1. N and Mn Content of Wheat Grain

The impact of weather conditions on the nitrogen content in the years of the study was different than that on the grain yield (it was the highest in 2016 and the lowest in 2014) (Table 4). The nitrogen fertilisation at 150 and 200 kg (without Mn) increased the N content in grain of statistically significant value compared to the level on the plots without fertilisation (by 20.2 and 30.1%, respectively).

The highest Mn content was determined in grain obtained in 2016, and it was higher by 37% on average compared with the Mn content in 2014 and 2015 (Table 5). The lowest average Mn content was found in the grain harvested on non-fertilised plots. However, no statistically significant differences in the element content were demonstrated compared with the grain obtained from plots fertilised with 150 kg N and 150 kg N + 0.5 kg Mn. As a result of the combined effect of the fertilisation and the study years, the application of 150 kg N ha^{-1} + 1.5 kg Mn ha^{-1} increased the Mn content in grain compared with the plots fertilised by nitrogen at the same rate without Mn. The application of 200 kg N ha^{-1} with 1.5 kg Mn ha^{-1} in 2016 increased the Mn content statistically significantly compared with the variant of 200 kg N ha^{-1} without manganese.

Table 4. Content of N in grain after application of N and Mn fertilisers, g kg^{-1}.

Treatment	Year			Average for Treatment
	2014	2015	2016	
Without fertilisation	12.27 h	15.25 gh	21.34 bc	16.3 d
150 kg N ha^{-1}	15.63 g	19.20 c–f	23.84 ab	19.6 c
150 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	16.11 fg	19.47 c–e	25.76 a	20.4 a–c
150 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	15.84 g	19.95 c–e	23.89 ab	19.9 bc
200 kg N ha^{-1}	17.76 d–g	20.85 b–d	25.07 a	21.2 ab
200 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	17.49 e–g	20.96 bc	26.13 a	21.5 a
200 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	17.49 e–g	21.33 bc	24.94 a	21.2 ab

a,b,c,d,e,f,g,h—values followed by the same letters do not differ significantly in Tukey's (HSD) test ($p < 0.05$).

Table 5. Content of Mn in grain, after application of N and Mn fertilisers, mg kg^{-1}.

Treatment	Year			Average for Treatment
	2014	2015	2016	
Without fertilisation	19.83 l	20.37 l	29.70 d–f	23.30 b
150 kg N ha^{-1}	20.90 kl	22.57 kl	30.20 de	24.56 b
150 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	22.80 kl	21.83 kl	29.03 d–g	24.56 b
150 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	27.53 d–h	25.83 f–j	34.43 bc	29.97 a
200 kg N ha^{-1}	25.23 g–j	27.53 d–h	30.83 cd	27.87 a
200 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	23.87 h–l	24.80 h–k	35.03 b	27.90 a
200 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	26.23 e–i	24.43 h–k	38.50 a	29.72 a

a,b,c,d,e,f,g,h,i,j,k,l—values followed by the same letters do not differ significantly in Tukey's (HSD) test ($p < 0.05$).

3.2. Infestation with Fusarium spp.

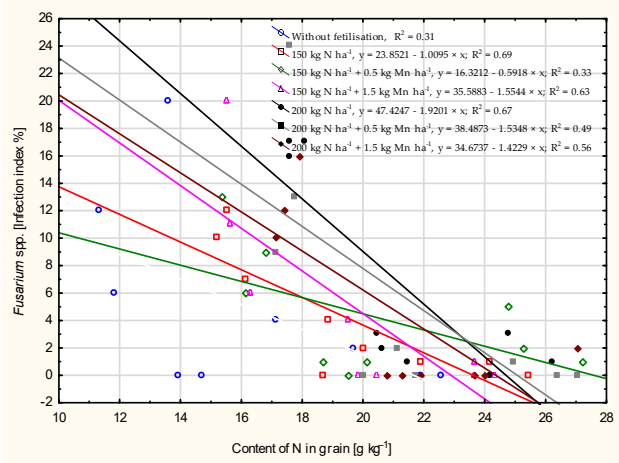
Fungi of *Fusarium* spp. occurred in wheat heads with the greatest intensity in 2014. However, those were not the amounts of fungi that might pose a considerable threat to the wheat head condition (Table 6). The higher intensity of *Fusarium* head blight was caused by favourable conditions for plant growth, manifesting themselves in higher stand density, which caused the deterioration in the phytosanitary conditions at the end of the growing period. The infestation was under the harmfulness threshold in the other two years of the study. No impact of the nitrogen or manganese fertilisation on the *Fusarium* head blight symptom occurrence on wheat heads was demonstrated during the study years.

Table 6. Infestation with *Fusarium* spp. after application of N and Mn fertilisers, infection index %.

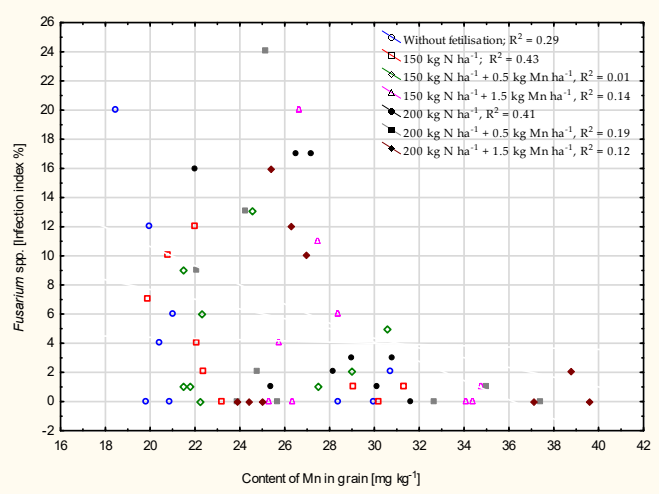
Treatment	Year			Average for Treatment
	2014	2015	2016	
Without fertilisation	12.67 a	1.33 c	0.67 c	1.89 a
150 kg N ha^{-1}	9.67 a–c	2.00 c	0.67 c	4.11 a
150 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	9.33 a–c	0.67 c	2.67 bc	4.22 a
150 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	12.33 ab	1.33 c	0.33 c	4.67 a
200 kg N ha^{-1}	16.67 a	2.00 c	1.33 c	6.67 a
200 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	15.33 a	0.67 c	0.33 c	5.44 a
200 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	12.67 a	0.01 c	0.67 c	4.44 a

a,b,c—values followed by the same letters do not differ significantly in Tukey's (HSD) test ($p < 0.05$).

The coefficient of determination (R^2) reveals that over 50% of the infestation severity caused by *Fusarium* spp. was influenced by the nitrogen content of grain in plots treated with nitrogen fertilisation at rates of 150 and 200 kg N. (R^2 = 0.69 and r^2 = 0.67), as well as Mn at 1.5 kg (R^2 = 0.63 and R^2 = 0.56) (Figure 1). *Fusarium* head blight was not affected by the Mn content of the grain.



(**a**)



(**b**)

Figure 1. Dependence of intensity of Fusarium head blight on content of N (**a**) and Mn (**b**) in grain (calculated at significance $\alpha = 0.05$).

3.3. Mycotoxin Content

Four mycotoxins were found in wheat grain: deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEA) and moniliformina (MON) (Tables 7–10). The weather conditions during the study years were found to have a varied impact on cereal grain contamination with mycotoxins produced by *Fusarium*. The largest amounts of DON and NIV were found during the first growing season (2014), whereas the largest amounts of MON were found in 2015, and of ZEA, they were found in 2016.

Table 7. Concentrations of deoxynivalenol (DON) in winter wheat grain after application of N and Mn fertilisers, μg g^{-1}.

Treatment	Year			Average for Treatment
	2014	2015	2016	
Without fertilisation	1053.04 bc	1302.87 a	944.55 cd	1100.15 ab
150 kg N ha^{-1}	1118.26 a–c	1125.58 a–c	1144.2 a–c	1119.42 a
150 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	1307.98 a	944.71 cd	579.20 fg	943.97 cd
150 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	807.79 de	1239.63 ab	638.45 e–g	895.29 d
200 kg N ha^{-1}	1055.67 bc	666.78 e–g	1329.6 a	1008.47 bc
200 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	731.13 e–g	541.62 g	762.97 d–f	678.57 e
200 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	826.86 de	531.83 g	687.26 e–g	682.65 e

a,b,c,d,e,f,g—values followed by the same letters do not differ significantly in Tukey's (HSD) test ($p < 0.05$).

Table 8. Concentrations of Nivalenol (NIV) in winter wheat grain after application of N and Mn fertilisers, μg g^{-1}.

Treatment	Year			Average for Treatment
	2014	2015	2016	
Without fertilisation	559.26 ab	326.39 ef	60.30 ij	315.31 b
150 kg N ha^{-1}	621.02 a	415.65 d	77.69 ij	371.46 a
150 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	521.36 bc	303.25 f	59.44 ij	294.68 b
150 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	454.92 cd	211.06 g	87.37 hi	251.12 c
200 kg N ha^{-1}	478.65 b–d	392.84 de	65.84 ij	312.44 b
200 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	311.31 ef	180.23 gh	45.97 ij	179.17 d
200 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	188.47 g	94.42 hi	0.01 j	94.30 e

a,b,c,d,e,f,g,h,i,j—values followed by the same letters do not differ significantly in Tukey's (HSD) test ($p < 0.05$).

Table 9. Concentrations of Zearalenone (ZEA) in winter wheat grain after application of N and Mn fertilisers, μg g^{-1}.

Treatment	Year			Average for Treatment
	2014	2015	2016	
Without fertilisation		8.78 d–f	23.28 b	14.81 ab
150 kg N ha^{-1}	10.41 de	7.60 d–f	30.10 a	16.03 a
150 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	13.21 cd	7.94 d–f	12.27 d	11.14 bc
150 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	8.48 d–f	5.55 e–g	8.21 d–f	7.41 c
200 kg N ha^{-1}	8.77 d–f	2.93 fg	30.99 a	14.23 ab
200 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	5.77 e–g	4.47 e–g	18.39 bc	9.55 bc
200 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	3.15 fg	0.75 g	7.93 d–f	3.94 d

a,b,c,d,e,f,g—values followed by the same letters do not differ significantly in Tukey's (HSD) test ($p < 0.05$).

Table 10. Concentrations of moniliformina (MON) in winter wheat grain after application of N and Mn fertilisers, μg g^{-1}.

Treatment	Year			Average for Treatment
	2014	2015	2016	
Without fertilisation	24.31 g–j	47.75 b–d	58.82 ab	43.63 a
150 kg N ha^{-1}	39.68 c–g	71.70 a	28.22 e–i	46.53 a
150 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	19.29 h–j	56.64 a–c	18.61 h–j	31.52 bc
150 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	24.43 g–j	26.53 f–i	38.59 d–g	29.85 bc
200 kg N ha^{-1}	28.35 e–i	44.28 b–e	32.05 d–h	34.89 b
200 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	45.81 b–e	12.26 i–k	19.48 h–j	25.85 c
200 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	43.71 b–f	0.01 k	8.15 jk	17.29 d

a,b,c,d,e,f,g,h,i,j,k—values followed by the same letters do not differ significantly in Tukey's (HSD) test ($p < 0.05$).

The application of nitrogen at 150 kg (without Mn) resulted in the presence of the highest grain mycotoxin content. However, the differences were not statistically significant compared with the plot with no fertilisation in the case of MON content. Supplementation of nitrogen fertilisation with manganese reduced the number of mycotoxins in wheat grain, which was confirmed by statistical analysis.

The DON content in the fertilised plot in which 150 kg N and 0.5 kg Mn were applied was determined at 93% by the N content of the grain (Figure 2). The calculated coefficient of determination (R^2) shows that the NIV level in all the variants was determined by the nitrogen content of the grain (from 88% to 96%). The coefficient of determination (r^2) for the level of MON was higher than 50% at the no-fertilisation plot and at the plot fertilised with 200 kg N and 1.5 kg Mn. The ZEA level was determined (from 64% to 72%) by the N content of grain at the no-fertilisation plot and in the following variants: 150 kg N, 200 kg N and 200 kg N + 0.5 kg Mn.

The inclusion of 150 kg of nitrogen along with manganese (0.5 and 1.5 kg) resulted in the Mn content of grain accounting for 56–68% of the variation in the DON level (Figure 3). The NIV level was the most weakly determined when 150 kg N and 1.5 kg Mn were applied ($R^2 = 0.37$) and the most strongly determined when 150 kg N was applied ($R^2 = 0.91$). The MON level was determined by the Mn content in the no-fertilisation plot (50%) and in the plot where 150 kg N and 1.5 kg Mn were applied (56%). The ZEA level was determined by the Mn content in the no-fertilisation plot (82%) and in the plot where 150 kg N (90%), 200 kg N + 0.5 kg Mn (94%) and 200 kg N + 1.5 kg Mn (66%) were applied.

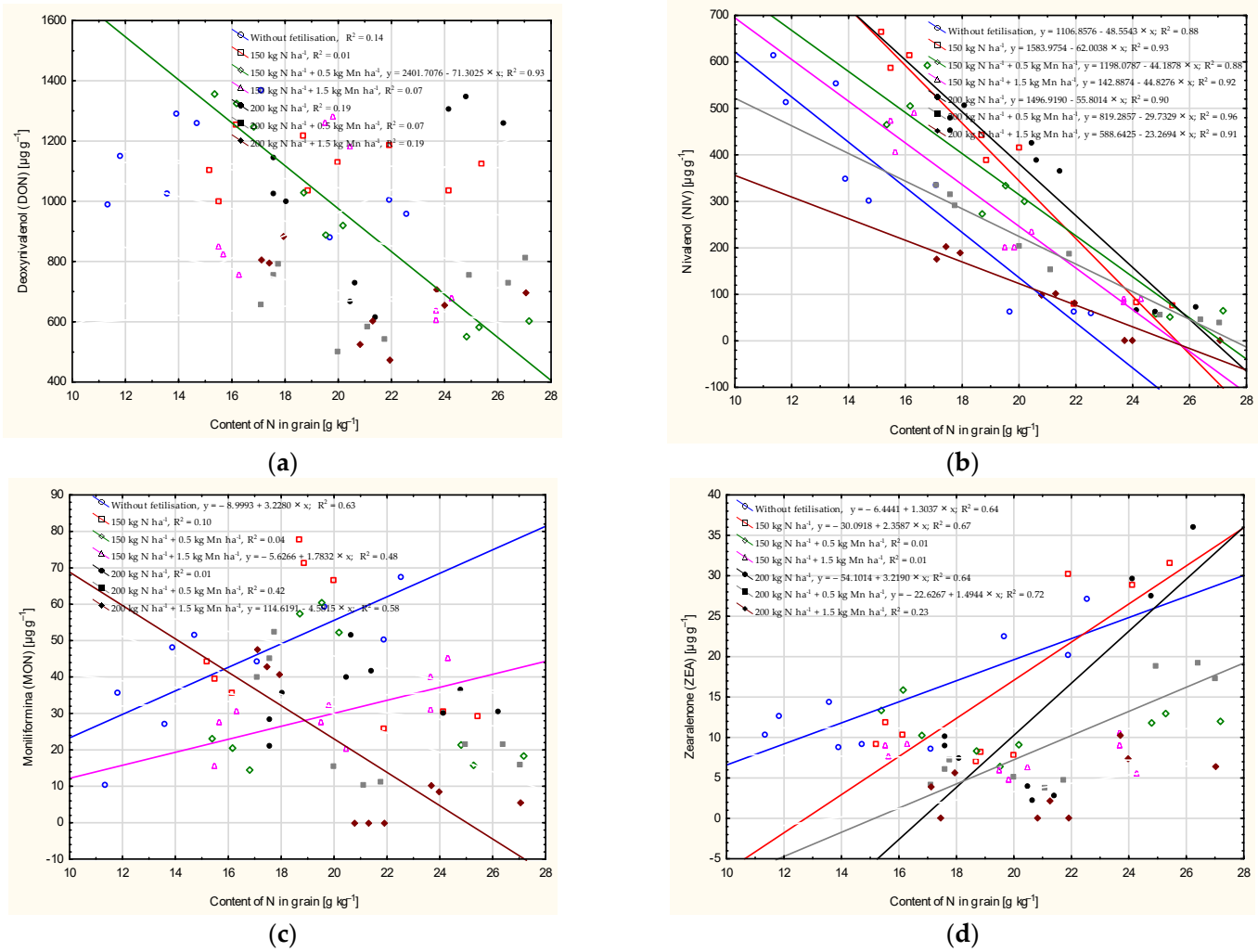


Figure 2. Dependence of content of N on the number of mycotoxins in wheat grain: deoxynivalenol (DON) (**a**), nivalenol (NIV) (**b**), moniliformin (MON) (**c**), zearalenone (ZEA) (**d**), calculated at significance $\alpha = 0.05$).

The DON level was determined at 71% by the occurrence of *Fusarium* spp. following the application of 200 kg N ha^{-1} and 1.5 kg Mn ha^{-1} (Figure 4). The coefficient of determination for the NIV level exceeded 50%, except in plots where 150 kg N ha^{-1}, 0.5 kg Mn ha^{-1} ($R^2 = 0.35$) and 200 kg N ha^{-1} were applied ($R^2 = 0.47$). The MON level was determined by grain contamination with *Fusarium* spp. in the no-fertilisation plot ($R^2 = 0.61$) following the application of 200 kg N ha^{-1} with Mn ($R^2 = 0.72$–0.86). The ZEA level was not determined by *Fusarium* spp. infestation.

3.4. Winter Wheat Grain Yield

The highest grain yield was obtained in 2014 (10.60 t ha^{-1}), and the smallest was obtained in 2016 (2.69 t ha^{-1}) (Table 11). The grain yield was mainly affected by the varied weather conditions during the wheat-growing periods. Thus, the low wheat grain yield in the last study year (2016) was a consequence of adverse thermal conditions in winter. Due to the high temperatures in November and December, the plants' vegetation did not stop, and the sudden temperature drop in early January, combined with no snow cover, froze part of the plants. Excessive rainfall during the initial period of the growing season (November 2015) hindered plant growth and left them ill-prepared for the winter season. Moreover, large amounts of rainfall at the end of the growing season (July and August) did

not favour the plant ripening and hindered the harvest. Irrespective of the rate, nitrogen or manganese fertilisation did not differentiate statistically significantly the wheat grain yield.

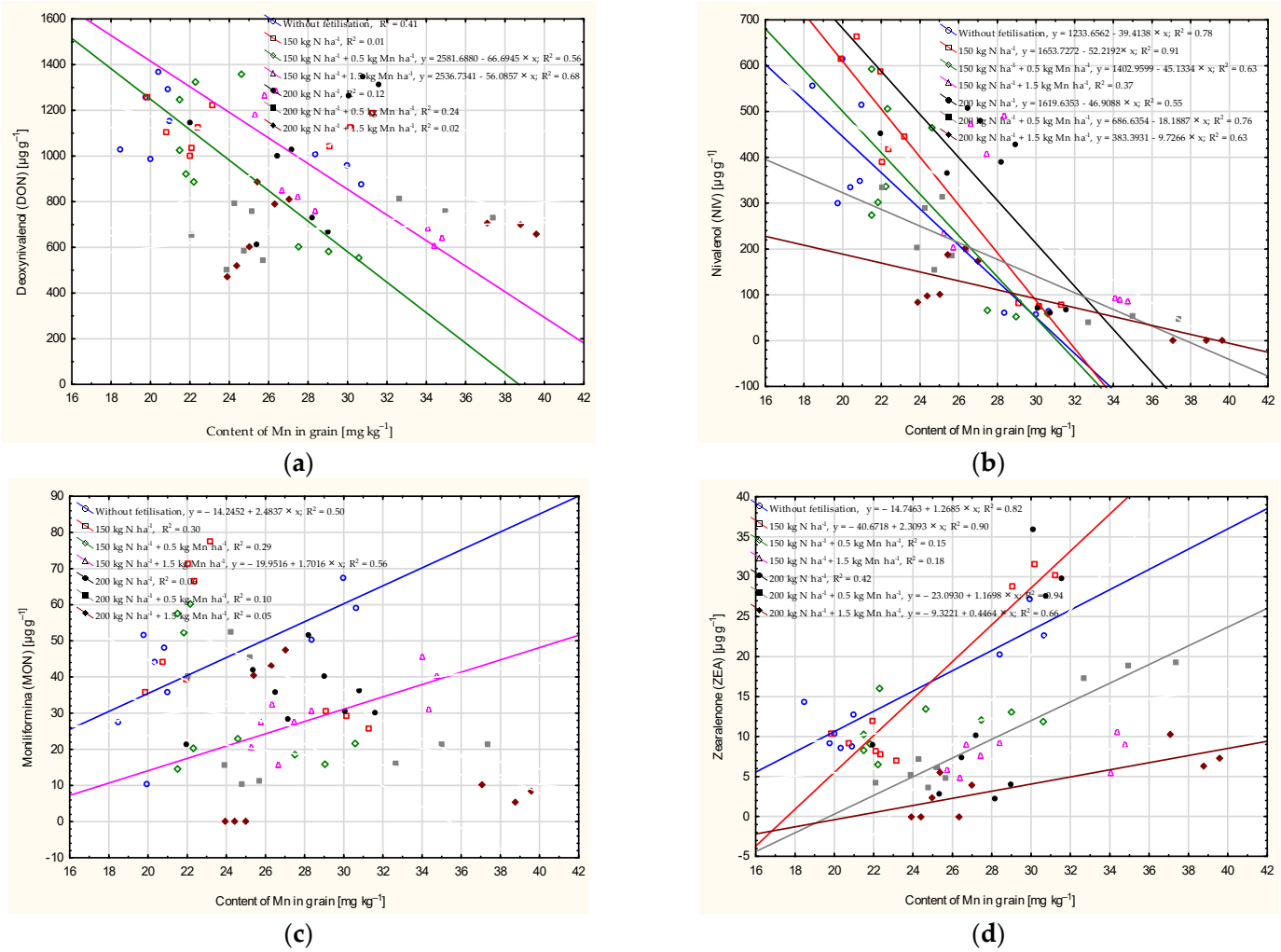


Figure 3. Dependence of content of Mn on the number of mycotoxins in wheat grain: deoxynivalenol (DON) (**a**), nivalenol (NIV) (**b**), moniliformin (MON) (**c**), zearalenone (ZEA) (**d**), calculated at significance $\alpha = 0.05$).

The coefficient of determination (R^2) shows that the grain yield was determined in most experimental plots by the *Fusarium* spp. infestation (except in the plot where 150 kg N ha^{-1} and 0.5 kg Mn ha^{-1} were applied) (Figure 5).

3.5. Principal Component Analysis (PCA)

The analysis of the impact of weather conditions (rainfall and temperature) and the use of nitrogen and manganese fertilisation in winter wheat cultivation on the content of N, Mn, grain yield, ear infection by *Fusarium* spp. and the content of mycotoxins in grain was complemented by the determination of the correlation between the above-mentioned factors. To this end, the principal component analysis (PCA) method was used to determine the links (the strength and direction of the correlation) between the measurement variables. PCA showed that the air temperature in the growing seasons (September–August) had an effect on grain yield (Figure 6). The fertilisation variant had an impact on the formation of nitrogen content in the grain. The year of cultivation and the amount of rainfall affected the content of manganese in grain. The performed PCA showed that the temperature (IV–VIII) had an effect on the infection of spikes by *Fusarium* spp. (Figure 7). During the growing

season in spring and summer, temperature and rainfall had no effect on the development of MON and DON mycotoxins.

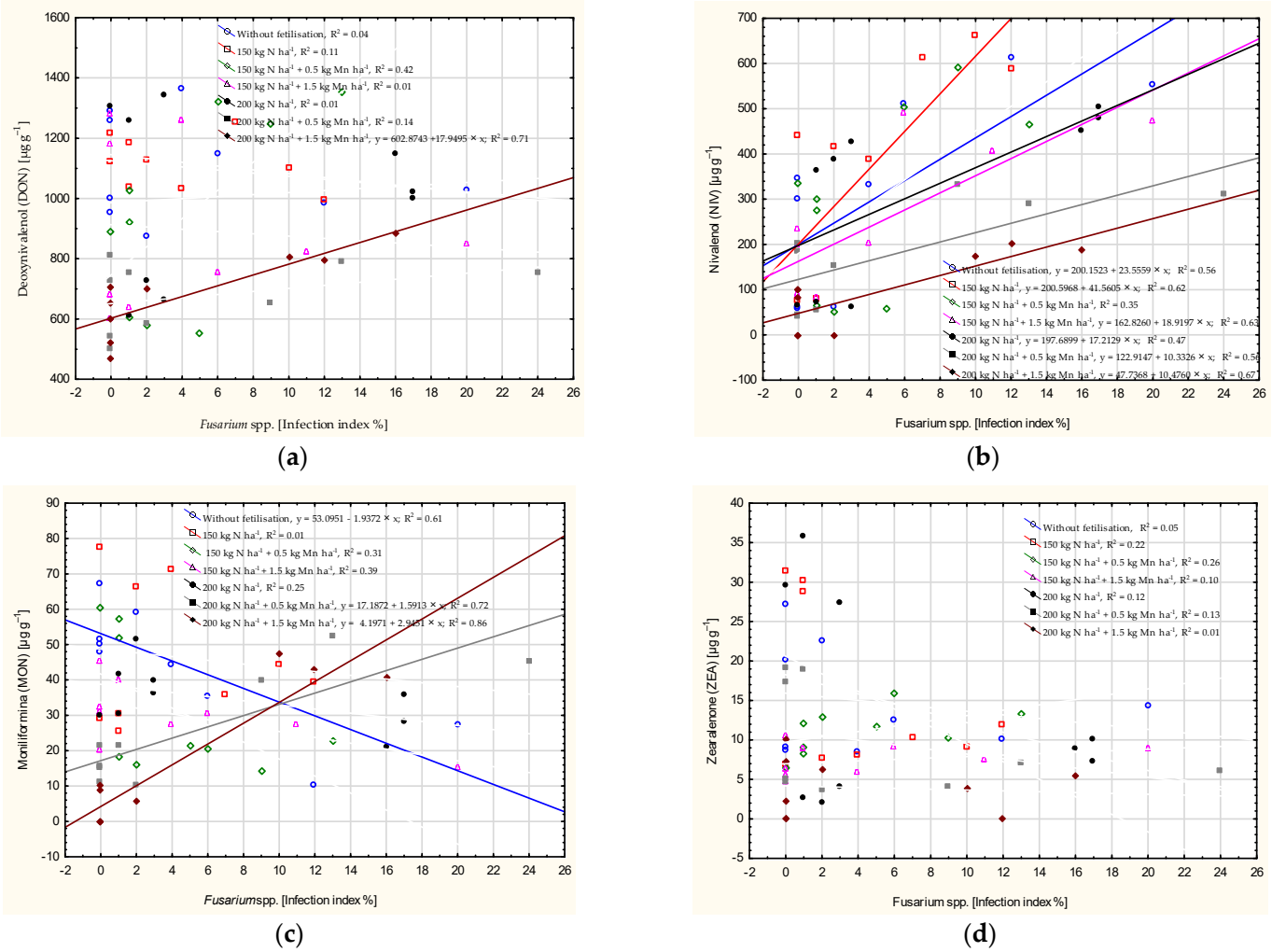


Figure 4. Dependence of intensity of Fusarium head blight on the number of mycotoxins in wheat grain: deoxynivalenol (DON) (**a**), nivalenol (NIV) (**b**), moniliformin (MON) (**c**), zearalenone (ZEA) (**d**), calculated at significance $\alpha = 0.05$).

Table 11. The grain yield after application of N and Mn fertilisers, t ha^{-1}.

Treatment	Year			Average for Treatment
	2014	2015	2016	
Without fertilisation	6.09 b	3.11 c–e	1.53 e	3.58 b
150 kg N ha^{-1}	10.95 a	6.19 b	2.92 c–e	6.69 a
150 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	11.32 a	7.08 b	2.99 c–e	7.13 a
150 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	11.23 a	5.80 bc	2.62 de	6.55 a
200 kg N ha^{-1}	11.25 a	6.73 b	3.03 c–e	7.00 a
200 kg N ha^{-1} + 0.5 kg Mn ha^{-1}	11.59 a	6.50 b	2.70 de	6.93 a
200 kg N ha^{-1} + 1.5 kg Mn ha^{-1}	11.76 a	5.57 b–d	3.07 c–e	6.80 a

a,b,c,d,e—values followed by the same letters do not differ significantly in Tukey's (HSD) test ($p < 0.05$).

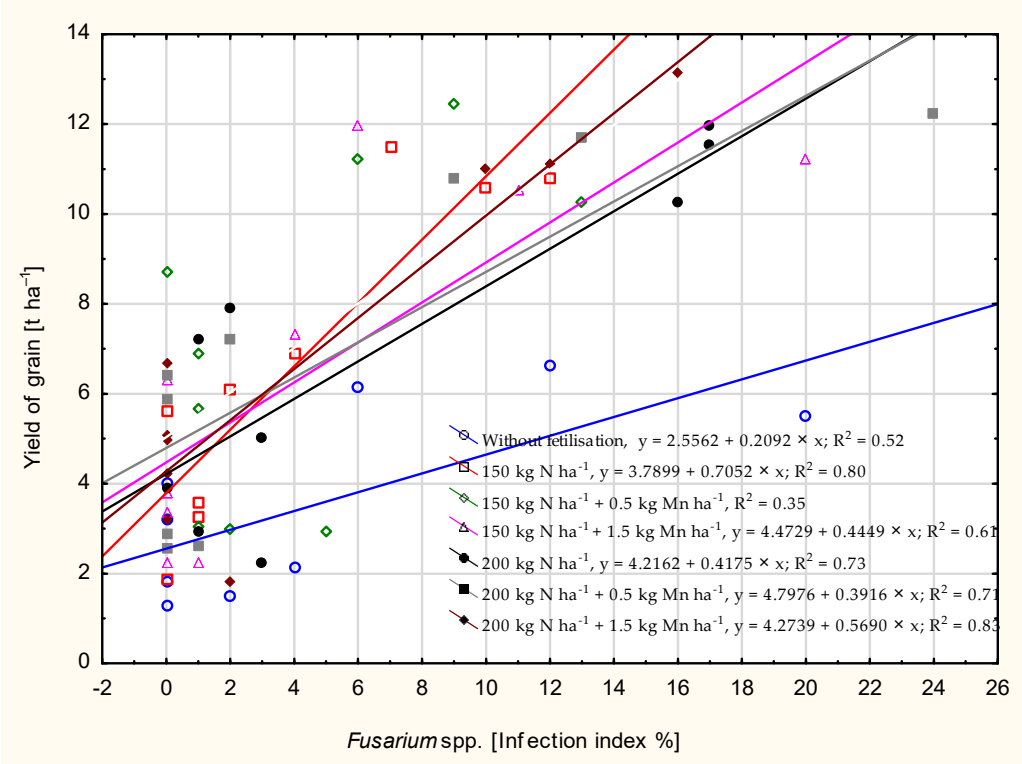


Figure 5. Dependence of intensity of *Fusarium* head blight on the grain yield, calculated at significance $\alpha = 0.05$).

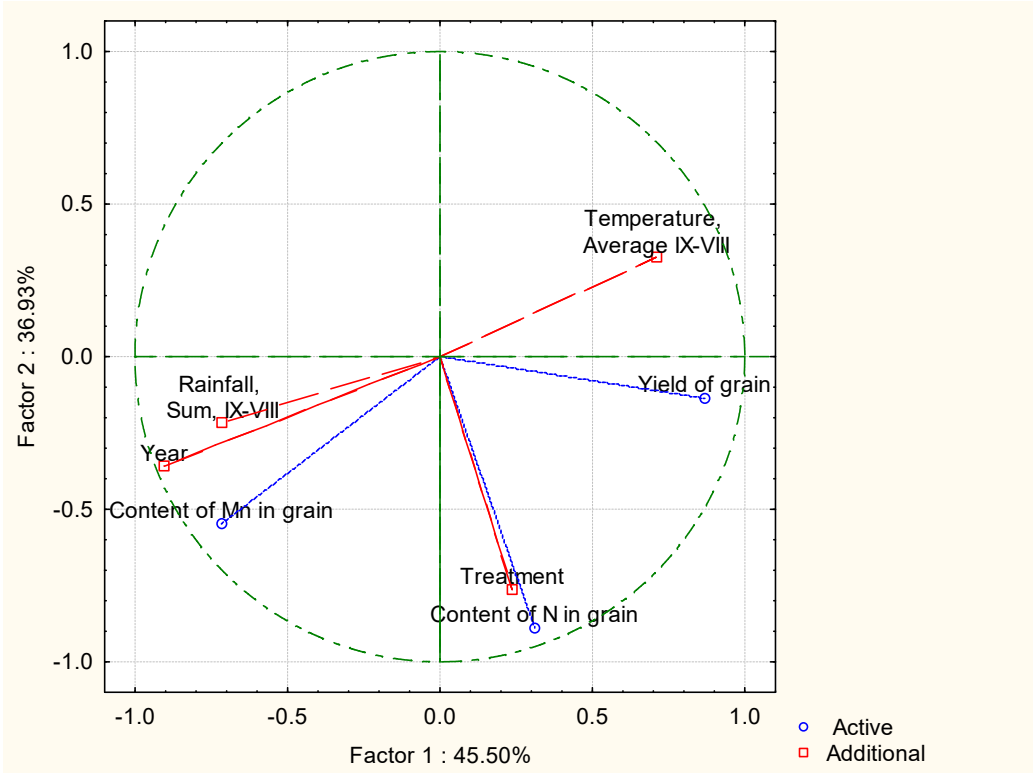


Figure 6. The correlation of the influence of weather conditions (rainfall and temperature IX–VIII) on the content of Mn, N and the yield of wheat grain.

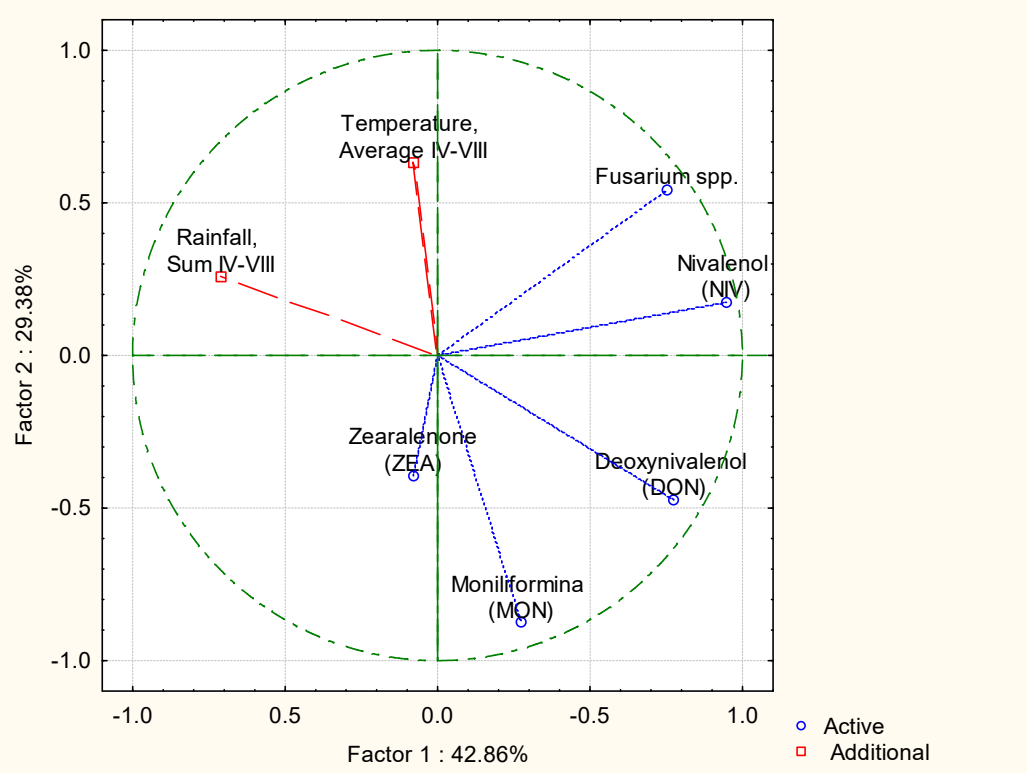


Figure 7. The correlation of the influence of weather conditions (rainfall and temperature, IV–VIII) on the intensity of Fusarium head blight and number of mycotoxins in wheat grain: deoxynivalenol (DON); nivalenol (NIV); moniliformin (MON); zearalenone (ZEA).

4. Discussion

4.1. N and Mn Content of Wheat Grain

The diversity of nitrogen and manganese content during the study years was an effect of varied yield (a higher yield in 2014 corresponded to a low N and Mn content, and a low grain yield in 2016 corresponded to a high N and Mn content of grain). The low nutrient content of grain in high-output cultivation is associated with intensive yield mass growth relative to mineral component absorption [35]. The effects of dilution of mineral components in the yield are presented in papers by Murphy et al. [36], Hussain et al. [37], Guttieri et al. [38] and Smith et al. [39] and not confirmed in studies by Shi et al. [40], Hamnér et al. [41] and Marles [42].

The nitrogen and manganese fertilisation as applied in the experiment conducted by these authors caused an increase in the nitrogen content of grain compared with the grain obtained in non-fertilisation plots. Nitrogen and manganese fertilisation did not have an equal impact on the manganese content of the grain. Mn is essential in N metabolism, and it takes part in nitrate reduction [43]. Shi et al. [40], Wojtkowiak et al. [1,44] and Jańczak-Pieniążek et al. [45] found that nitrogen fertilisers did not have an impact on the Mn content of winter wheat grain, and studies by Svecnjak et al. [46], Klikocka and Marks [47] and Dolijanović et al. [48] showed the opposite tendencies, i.e., that the manganese concentration increased with increasing the N rate.

4.2. Fusarium spp. Infestation and Mycotoxin Level

Fusarium head blight was determined by the habitat conditions during the crop-growing period. The weather conditions at the beginning of autumn in 2013 favoured a higher plant density and stronger tillering. These conditions during the final growing period, combined with increased humidity and temperature, resulted in a deterioration in the phytosanitary conditions, manifesting themselves in more intensive infestation with *Fusarium* spp. A lower plant density favours the creation of a beneficial microclimate, which

is important when higher rainfall is combined with higher temperatures [29]. This kind of microclimate boosts the growth of kernels, accelerates wheat head drying and shortens the time of exposure to infestation with *Fusarium* spp. According to Bryła et al. [16] and El Chami et al. [4], the development, growth and propagation of *Fusarium* in wheat depends on the amount of rainfall. Czaban et al. [13] and El Chami et al. [4] found infestation with *Fusarium* to be impacted by high rainfall during cereal blossoming. Fusarium head blight is favoured by long periods of high humidity, higher temperatures (between 15 and 30 °C) and the occurrence of air currents, disseminating fungi spores during and after the wheat-blossoming period [49].

Nitrogen or manganese fertilisation, as examined in a study conducted by the current authors, did not have an impact on the appearance of *Fusarium* spp. symptoms on wheat heads. However, the infestation intensity decreased with increasing nitrogen and manganese content. Infestation with *Fusarium* spp. depends on agronomic procedures, the effectiveness of the fungicides applied and the host's resistance [8]. Despite the association with *Fusarium* spp. infestation, the presence of mycotoxins is largely dependent on the land tilling systems, crop rotation, fertilisation and grain storage [50]. Balanced fertilisation, applied in proper form, can provide suitable nutrients and, therefore, make plants more resistant to pathogen infiltration [51]. Most studies dealing with *Fusarium* spp. infestation and grain contamination with mycotoxins focus on the impact of nitrogen fertilisation [52–54] Applying excessive amounts of nitrogen results in extended plant vegetative growth, making leaves more exposed to pathogens and the cellular wall thinner and more susceptible to penetration by fungi [55]. According to Lemmens et al. [22] and Piekarczyk i Lemańczyk [56], increasing nitrogen fertiliser rates had a significant impact on higher wheat infestation by *Fusarium*. The results of the experiments conducted by Aufhammer et al. [23], Krnjaja et al. [24] and El Chami et al. [4] did not show a significant impact of nitrogen fertilisation on infestation by *Fusarium* spp. and mycotoxin production in wheat grains.

Four mycotoxins were found in wheat grain: deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEA) and moniliformin (MON). The weather conditions were found to have a varied impact on the cereal grain contamination with mycotoxins produced by *Fusarium*. A study conducted by El Chami et al. [4] demonstrated a significant impact of the growing season on mycotoxin production due to higher rainfall during the blossoming period when the wheat head is the most susceptible to *Fusarium* infestation. Only the deoxynivalenol level, as determined in the current study, was correlated with *Fusarium* spp. infestation. The study conducted by Góral et al. [57] did not show any correlation between the *Fusarium* head blight index and the DON level, but a correlation was found to exist with contamination by NIV. Wickiel and Filoda [58] analysed the intensity of *Fusarium* head blight and DON level in spelt grain and found no correlation between the symptom intensity and the toxin level. Nitrogen application at 150 kg ha^{-1} (without Mn) in the current study caused higher grain contamination with toxins than at 200 kg ha^{-1} N. The results of an experiment conducted by Aufhammer et al. [23], Czaban et al. [16] and El Chami et al. [4] did not show a significant impact of nitrogen fertilisation on infestation by *Fusarium* spp. or mycotoxin production in wheat grains. As mentioned above, Lemmens et al. [22] linked mycotoxin occurrence with increased nitrogen fertiliser rates.

Supplementation of nitrogen fertilisation with manganese in this study reduced the number of mycotoxins in wheat grain. Micronutrients contribute to the development of plant resistance to pathogens [59]. Manganese is an important micronutrient for plant growth and development [60,61]. It plays some metabolic roles in various processes of the plant life cycle, such as photosynthesis, respiration and the uptake of reactive oxygen species [62,63]. The presence of Mn is important for building structures of plant tissues, which is a physical barrier for pathogens [30,31].

4.3. Grain Yield

Net nitrogen productivity varied widely during the study years, and weather conditions largely decided the effectiveness of nitrogen fertilisation [64]. The grain yield determined in the current study was mainly affected by the varied weather conditions during the wheat-growing periods. Neither nitrogen nor manganese fertilisation differentiated the wheat grain yield. It has been shown in many studies [65,66] that foliar application of small amounts of micronutrients (alone or in combination with other essential nutrients) can increase the yield and its components, thereby improving wheat growth and quality. The grain yield was correlated with *Fusarium* spp. infestation since *Fusarium* head blight has an adverse impact on wheat grain yield [67]. This disease, as examined by Spanic [68], reduced the grain yield in some wheat cultivars by up to 64%.

5. Conclusions

Wheat infestation by *Fusarium* spp. was determined by the habitat conditions during crop growth. The performed PCA showed that the temperature (IV-VIII) had an effect on the infection of spikes by *Fusarium* spp. During the growing season in spring and summer, temperature and rainfall had no effect on the development of MON and DON mycotoxins. Neither nitrogen nor manganese fertilisation affected the presence of *Fusarium* spp. symptoms on wheat ears, but the infestation intensity decreased with increasing nitrogen and manganese content in the grain. Four mycotoxins were found in wheat grain: deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEA) and moniliformin (MON). Only the deoxynivalenol level was correlated with *Fusarium* spp. infestation. Nitrogen application at 150 kg ha^{-1} (without Mn) caused more intensive grain contamination with toxins than at 200 kg ha^{-1} N. Supplementation of nitrogen fertilisation with manganese reduced the number of mycotoxins in wheat grain (with the exception of ZEA content, which was unproved by statistical means). The grain yield was mainly affected by the varied weather conditions during the wheat-growing periods. Neither nitrogen nor manganese fertilisation differentiated the wheat grain yield.

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