N-Stabilizer and Foliar Fertilizer Treatments Enhance Tolerance to Specific Pathogens in Maize (*Zea mays* L.)

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Summary

Presently, due to unpredictable climatic conditions crop cultivation has become extremely challenging. Adverse environmental factors, such as uneven precipitation distribution, frequent soil droughts, extreme temperature fluctuations all induce a disturbance in crop nutrient uptake, and the infections of pathogens in stressed crops pose an increased threat, as well Besides the fact that foliar fertilizer is energetically beneficial for crops, it also improves nutrient use and prevents nutrient deficiencies. Despite the regular and wide-range application of nitrogen (N) fertilizers, N-losses resulting from nitrate leaching are often considerable, which can be reduced by N-stabilizers providing sufficient available N-forms in the soil for crops during the critical growth stage. The present paper focuses on enhancing the natural tolerance of the maize (*Zea mays* L.) treated with N-stabilizer containing nitrapyrin and foliar fertilizer to the Fusarium and Aspergillus ear rot (*Fusarium verticillioides*; *Aspergillus flavus*) pathogens. Data suggest that the maize treated with both nitrapyrin and foliar fertilizer was the most resistant to the pathogens compared to untreated crops. The findings drive attention to the importance of optimal, balanced nutrient supply.

Key words

maize, ear infection, *Fusarium verticillioides*, *Aspergillus flavus*, nitrogen stabilizer, foliar fertilizer

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Introduction

In recent years, a growing body of scientific studies on sustainable crop protection and cultivation have indicated that due to climate change, crops face increasing challenges while the high demands of the population for quantity and quality of the yield have not diminished (Arora, 2019). Thus, developing agrotechnical solutions to mitigate stress factors on the crops is highly relevant. Several prior studies reported that appropriate and balanced nutrient supply supported the healthy condition of crops (Mengel and Kirkby, 2001; Kumar et al., 2018). However, it is important to note that this does not mean that most diseases can be controlled only by nutrient supply, it only highlights more clearly that professional nutrient supply must be an essential part of the integrated pest management in disease prevention and treatment (Reuveni and Reuveni, 1998; Singh and Gohain, 2017).

The health of maize is threatened by numerous pathogens, including toxigenic filamentous fungi, such as *Fusarium verticillioides* (*Hypocreales, Nectriaceae*), and *Aspergillus flavus* (*Eurotiales, Trichocomaceae*). Animals consuming feed contaminated with mycotoxins, as well as people who consume such animal products face medical risks (Garcia et al., 2012, Battilani et al., 2016). *F. verticillioides* has been in public knowledge for a longer time in link with the health protection of maize and it is considered to be one of the most dangerous field pathogens due to the production of the fumonisins (FB1, FB2, FB3) (Schulthess et al., 2002; Rosa Junior et al., 2019). In most cases, Fusarium ear rot shows clear symptoms: white-to pink or salmon-coloured mold at the ear tip (Wang et al., 2014). Insect activity is associated with fumonisin contamination as they disperse the fungus and provide routes for entry into the maize ear and kernels (Alma et al., 2005). A higher temperature during maturation and heavy rainfall creating humid conditions before harvest were reported to support infection and increase ear rot levels and fumonisin content at harvest (Fandohan et al., 2003; De la Campa et al., 2005). The effects of climate change are well reflected by the fact that the *A. flavus* has been known only as a pathogen of stored products. However, the fungus damages arable crops as well, as its spread and toxin production is favoured by the extremely hot, dry summers (Abbas et al., 2006, 2012; Guo et al., 2016; Maisello et al., 2019). Aflatoxins produced by the fungus (primarily B1) are the most important carcinogen secondary metabolites (Henry et al., 1999; Marchese et al., 2018). In most cases, infections are strongly linked to insect injuries (*Ostrinia nubilalis*, *Helicoverpa armigera*, *Diabrotica virgifera*), thus, appropriate timing of insecticide treatments has a key role. Symptoms of *Aspergillus* ear rot on maize are obvious: green or olive-coloured mold-covered surfaces can be seen on infected kernels (Thathana et al., 2017).

The relation between nutrient supply and pathogen infection is extremely complex (Huber and Graham, 1999; Huber et al., 2012). As a consequence of soil drought, nutrient uptake of crops is becoming energetically stressful, resulting in the increasing significance of foliar fertilizer (Fernández and Eichert, 2009). Although foliar fertilizers are not a part of everyday practice (Pecznik, 1976; Harder et al., 1982), the limited availability of nutrients in the soil prompts farmers to use additional foliar fertilizer treatment (Fernández and Eichert, 2009). Furthermore, it prevents latent or hidden nutrient deficiency diseases, enhancing the vitality and tolerance of crops. Foliar fertilization contributes to enhancing photosynthesis and respiration intensity, thus improving quantitative and qualitative parameters of the yield (Fageira et al., 2009).

Nitrogen (N) as one of the most essential nutrients in maize is a determining factor of crop cultivation as the N demand of maize is higher than any other nutrient (Forde and Clarkson, 1999; Pakurár et al., 2004). Despite the widespread practice of N-fertilization, crops often show symptoms of severe N-deficiency, even though the divided N application aims to reduce N-loss mainly resulting from nitrate leaching that contaminates underground waters (Huang et al., 2017; Dimpka et al., 2020). Nitrate leaching has the greatest responsibility for N-loss (10-50%) as it moves freely in the soil, which can be controlled by N-stabilizer treatment (Futó et al., 2016). N-stabilizer containing nitrapyrin blocks the activity of *Nitrosomonas* species involved in the nitrification process in the soil and is responsible for the conversion of ammonium to nitrite ions, providing stable ammonium-N forms available to crops for a longer period. In this way, N-loss resulting from nitrate leaching can be reduced (Vanelli and Hopper, 1992; Papp, 2014; Degenhardt et al., 2016; Futó et al., 2016; Woodward et al., 2019). Nitrapyrin contributes to improving the efficiency of N-fertilization, leads to healthier and more resistant crop vegetation, improved yield, and besides, it significantly reduces the environmental pollution resulting from nitrate-leaching (Randall et al., 2003a; Papp, 2014).

For maize nutrient supply, particular attention should be paid to magnesium (Mg) as the compound of chlorophyll responsible for photosynthesis, and to zinc (Zn) that contributes to the optimal protein synthesis. In the case of Zn deficiency, tolerance of crops becomes poor and increased risks inherent in the infection of pathogens should be expected (Cakmak and Yaizei, 2010). Some studies reported that optimal Zn supply improved drought tolerance, which contributes to the alleviation of *Aspergillus* induced fungal infection (Cakmak et al., 1989; Cakmak et al., 1994; Datnoff et al., 2006; Gérardeaux et al., 2009). As a result, theory cannot be excluded as the maize treated with nitrapyrin in order to improve the N-utilization, and with foliar fertilizer jointly resulting in better nutrient supply may contribute to enhance pathogen control of the crops.

The study aims to examine the relationship between nutrient supply and tolerance of maize (*Zea mays* L.) to artificial ear infection of *F. verticillioides* and *A. flavus* pathogenic filamentous fungi. To enhance the efficient utilization of N and other nutrients, N-stabilizer containing nitrapyrin and foliar fertilizer applications were performed in the scope of the presented experiment.

Materials and Methods

Design of the Experiment

The field experiment was conducted in 2020, in the Demonstration Garden of the Institute of Plant Protection (University of Debrecen, Debrecen, Hungary; 47°33'07.9"N, 21°36'03.0"E). The test crop was feed maize (*Zea mays* L.) hybrid (Armagnac, FAO 490, Kite Zrt, Hungary). Maize was sown on 23 April (crop density: 72,000 crops ha^{-1}). No fungicide and insecticide treatments were conducted during vegetation. N-stabilizer containing nitrapyrin (N-Lock™ Max, Corteva Agriscience, Wilmington, USA) was sprayed on 13 June, at a

25°C soil temperature, with 1.7 L ha^{-1} dose (300 g L^{-1}). Within two weeks 45 mm precipitation was received that activated soil-applied nitrapyrin (Hungarian Meteorological Service [HMS], 2020). Foliar fertilizer treatment was performed with 10 L ha^{-1} dose on 29 June, at the stage of 8 leaves of the maize (BBCH 17). The nutrient content of the foliar fertilizer is shown in Table 1. The amount of organic manure applied to the soil was 25 t ha^{-1}.

The efficiency of nitrapyrin and foliar fertilizer treatments were followed up with measurements of soil nitrate content, relative chlorophyll content of the maize leaves. Furthermore, the accurate nutrient content of leaves was determined by laboratory leaf analysis. The treatments (control: C; foliar fertilizer: F; nitrapyrin: N; nitrapyrin + foliar fertilizer: N+F) were designed in four-time repetitions and located in small plots. Each plot was 25 m^2 in size.

Table 1. Composition of the applied foliar fertilizer

Compound	mg L^{-1}
Total nitrogen (N)[a]	80,000
Phosphoric anhydride (P_2O_5)	70,000
Potassium oxide (K_2O)	70,000
Sulphur trioxide (SO_3)	70,000
Boron (B)[b]	127
Copper (Cu)[b]	162
Iron (Fe)[b]	284
Manganese (Mn)[b]	265
Zinc (Zn)[b]	38

[a] – urea nitrogen; [b] – EDTA chelated

Climatic Condition of the Year

The vegetation period of 2020 was characterized by an extremely uneven distribution of precipitation. The initial development of the maize was significantly impeded by the severe drought that lasted until the end of May in most parts of the country (only 51.7 mm of rain fell in April and May). The drought-induced soils were only alleviated by the lower rainfall in June, thus the growth of maize was still delayed. Intensive growth started in mid-June, when a series of rainfalls has begun (June: 117.9 mm; July: 96.7 mm precipitation), exposing several areas to inland water. During the tasselling period, the optimal amount of precipitation supported yield production. The general Growing Degree Days (GDD) was 1,574°C, which is 10-50 degrees lower than in the year 2019. However, compared to the long-term average, the excess is 30-70 degree-days (HMS, 2020).

Soil Characteristics of the Experimental Field

Soil analysis was performed by accredited HL-LAB Environmental and Soil Testing Laboratory (Debrecen, Hungary). The soil type of the experiment was loam. Plasticity index according to Arany (K_A) was 38 (loam), which was adopted to describe the soil texture and thus it indicates that the soil in question is sand (Hungarian Standards Institution [HSI], 1978a; Füleky and Vicze, 2007), with an average pH_{KCL} 6.83 value (slightly acidic soil; HSI, 1978b). Its humus content is 2.91%, carbonated lime content is 0.9% (lacking in calcium). AL-soluble P_2O_5 content is 481 mg kg^{-1} (HSI, 1999a), AL-soluble K_2O is 2,010 mg kg^{-1} (HSI, 1999a). KCl-soluble $N\text{-}NO_3+NO_2$ (all nitrate + nitrite; HSI, 1999b) content is 2.16 mg kg^{-1}.

Soil Nitrate Measurements

Soil samples were taken from the 0-30 cm depth randomly along a "W" line, taking into account the heterogeneity. Twelve soil samples were taken from targeted areas 6 times, approx. every 2-3 weeks depending on weather conditions as can be seen in Fig. 1. The soil nitrate content was measured with Nitrat 2000 soil kit (Stelzner, Germany) according to the steps recommended by the manufacturer.

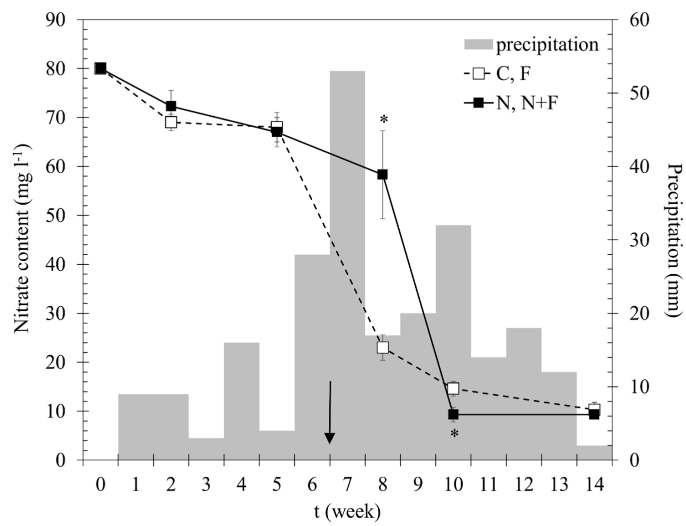

Figure 1. Nitrate content changes in soils depending on weekly precipitation totals. C: untreated control; F: foliar fertilizer; N: nitrapyrin; N+F: nitrapyrin and foliar fertilizer; The black arrow indicates the date of nitrapyrin application. Asterisks (*) denote statistically significant differences (Student's t-test, P ≤ 0.05) between nitrapyrin treated and untreated soils

Chlorophyll Measurement

Chlorophyll measurement was performed on 6 July, one week after foliar fertilization. The chlorophyll content of the maize leaves was determined using a SPAD-502 chlorophyll meter (Konica Minolta, Japan), which is based on two light-emitting diodes and a silicon photodiode receptor (Uddling et al., 2007). SPAD readings were calculated based on two transmission values, the 650 nm red light, which is absorbed by the chlorophyll, and 940 nm infrared light, at which no chlorophyll absorption occurs (Xiong et al., 2015). The measurement itself is simple as the device is a portable leaf-clip that must be clamped over the leaf tissue. The sensor generates within a few seconds a unitless SPAD reading (from 1 to 100) to indicate the index of chlorophyll *a* and chlorophyll *b* in thylakoid membrane in the leaf mesophyll chloroplasts (Konica Minolta, 1989; Kandel, 2020). Thus, this reading is proportional to the amount of chlorophyll present in the leaf tissue (Castelli et al., 1996; Uddling et al., 2007; Ling et al., 2010; Sowiński et al., 2018). Since also adult leaves (close to ears) and young (top) leaves provide important information related to the health condition, five SPAD meter readings were taken on each leaf (between the midvein and the leaf margin) and the mean of the measurements was retained as a value. Sampling units were defined randomly between each treatment.

Leaf Analysis

Twenty samples close to the ears (adult leaves) were collected in each treatment for nutrient leaf analysis. Sampling of adult leaves was timed 10 days after foliar fertilization to ensure that nutrients were utilized by the treated crops. Since nutrient analysis requires sufficiently large tissue samples (approx. at least double handful size), the whole part of adult leaves was removed by cutting them. Sampling units were defined randomly between each treatment. Accredited laboratory leaf analysis was performed by HL-LAB Environmental and Soil Testing Laboratory (Debrecen, Hungary). During the process, SLW 240 drying oven (Pol-Eko-Aparatura, Poland) was used for the preparation of samples from which the extraction was performed with HNO_3-H_2O_2 blend with MARS 6 microwave digester (CEM Corporation, USA). The N content of the extracts was determined on UDK 139 Semi-Automatic Kjeldahl Distillation Unit (Velp Scientifica Srl, Italy) and the other nutrients were determined on iCAP 6300 Radial View ICP-OES spectrometer (Thermo Fisher Scientific, USA).

Artificial Infection

For the artificial infection of the maize ear, pure cultures of *F. verticillioides* MRC 826 (NCAIM F.00935) and *A. flavus* NVK 20 (NCAIM F.00048) strains were used. Fungal strains were provided by the National Collection of Agricultural and Industrial Microorganisms ([NCAIM] Faculty of Food Sciences, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary). To prepare inocula, fungi were grown on Potato Dextrose Agar medium (Beever and Bollard, 1970; Merck Millipore, Germany) for 7 days at 25°C (*F. verticillioides*) and 37°C (*A. flavus*). Conidiospores were scrapped in sterile water, filtered through a sterile Miracloth tissue (Merck Millipore, Germany) and then counted with a haemocytometer. During inoculation in case of both cultures, 1 mL of spore suspension (5×10^6 spore) was injected into the maize silk channel using sterile syringes (Chirana T. Injecta, Slovakia). For *F. verticillioides*, inoculation was performed on 31 July (tasselling stage), and for *A. flavus*, on 18 August (during severe insect injuries). Since both microorganisms have potential risks to health and the environment, the inoculation procedure was performed by wearing well-fitting, N95 face mask, protective glasses, overalls and gloves. Sterile needles were used for each inoculation. Infected ears were covered with plastic bags after infection for seven days to maintain humidity which creates favourable conditions for the fungal infection. After the seventh day, plastic bags were removed and replaced with waterproof brown-paper tassel maize bags until the harvest on 15 September (Zuber et al., 1978). The infection degree was evaluated as a percentage (%) that was defined from the number of infected kernels (that showed visible symptoms) counted per maize ear.

Statistical Analysis

The statistical analysis was conducted by the R programming language (v4.0.2; R Core Team, 2020) and "agricolae" R package (v1.3-3; De Mendiburu, 2020). Student's t-test and its non-parametric version (Mann-Whitney U test) and Duncan's multiple comparison test were used to examine the means at a significance level of $P \leq 0.05$.

Results And Discussion

The effect of nitrapyrin treatment was observed by nitrate content changes in treated and control soil. Since nitrapyrin application was the same in "N" (nitrapyrin) and "N+F" (nitrapyrin + foliar fertilizer) treated fields and changes of nitrate content in the soil are not influenced by the foliar fertilizer (F) treatment, results of nitrate measurements obtained from the control (C) field include the results of foliar fertilizer (F) treatment and results obtained from "N" (nitrapyrin) treated field include the results of "N+F" (nitrapyrin and foliar fertilizer) treatment (Fig. 1). Since nitrapyrin was sprayed in the middle of June, soil temperature was relatively warm (25°C). Several prior studies on the impact of temperature on the nitrifying bacteria have reported that the activity of *Nitrosomonas* sp. increases as temperature elevates (Watson et al., 1981; Taylor et al., 2019). Chen et al. (2018) have also revealed that *Nitrosomonas* sp. were more active at 20°C. Thus, it can be concluded that the inhibitory effect of nitrapyrin acting on *Nitrosomonas* sp. was greater as it resulted in more NO_3-N (nitrate) forms in the nitrapyrin treated soil (N) compared to the control field (C), which provided better condition for the healthy development of maize during the rapid, high N-demanding growth stage. However, this result was not consistent with our hypothesis and prior studies that due to the nitrification delaying effect of nitrapyrin (Touchton et al., 1978), less nitrate concentration is expected in the treated soil as nitrapyrin increases ammonium retention and inhibits nitrification (Randall et al., 2003b; Randall and Vetsch, 2005; Ma et al., 2013; Sun et al., 2015; Zhang et al., 2015; Giacometti et al., 2020;). In the present research, at week 8 post-sowing, the nitrate content in the control, nitrapyrin-free (C; F) soils was significantly reduced compared to the nitrapyrin treated (N; N+F) soils (Fig. 1). It is more than possible that the continuous, heavy rainfall explains the decrease resulting in increased nitrate leaching in the near-surface soil. This agrees with several prior studies emphasizing the major impact of precipitation on nitrate leaching (Randall and Mulla, 2001; Schwenke and Haigh, 2016; Iqbal et al., 2017; Bowles et al., 2018; Martinez-Feria et al., 2019). The obtained results suggest that nitrapyrin provides more available N-forms in the soil creating more favourable conditions for the healthy growth and enhances N-use efficiency of the maize.

The effectiveness of the foliar fertilizer and nitrapyrin treatments were monitored by measuring the amount of relative chlorophyll in maize leaves correlating with the general health status of the crops, photosynthesis activity and N supply (Sowiński et al. 2018). Data obtained indicate that maize treated with nitrapyrin (N; N+F) showed the highest chlorophyll concentration, where the difference was significant for both adult and young leaves compared to the control (C) treatment (Fig. 2).

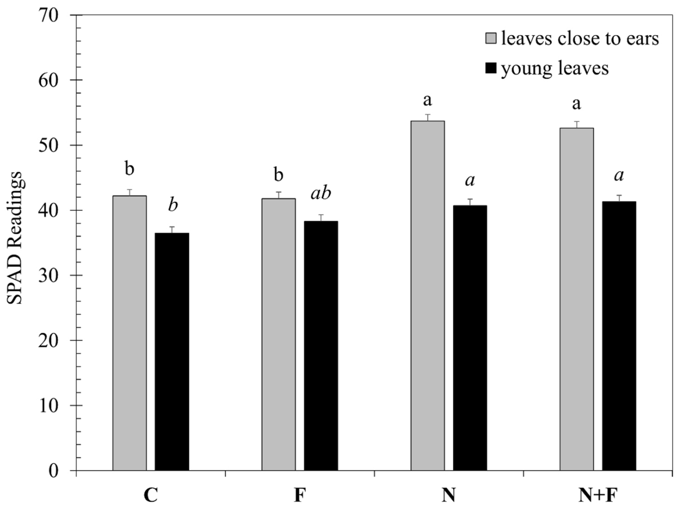

Figure 2. Changes in relative chlorophyll content due to each treatment. C: untreated control; F: foliar fertilizer; N: nitrapyrin; N+F: nitrapyrin and foliar fertilizer; Different lowercase letters denote statistically significant differences (Duncan's multiple comparison test, P ≤ 0.05) between treatments

Since no statistically significant difference was shown between the chlorophyll content of the control (C) and foliar fertilizer (F) treatment, it can be concluded that chlorophyll content increase was mainly influenced by the conditions of more favourable N uptake for the maize provided by nitrapyrin treatment.

This finding is in accordance with several prior studies (Singh and Nelson, 2019; Nozari et al., 2020; Ren et al., 2020; Taherianfar et al., 2020) that reported on increased chlorophyll amount and higher SPAD readings due to nitrapyrin. Besides, previous studies on foliar fertilizer treatment also emphasized its effect on enhancing photosynthesis activity of crops, thus increasing crop yields and quality (Harder et al., 1982; Tejada and Gonzalez, 2004; Abbas and Ali, 2011). Similar conclusions were drawn by our previous research when foliar fertilization significantly increased SPAD values (Rácz and Radócz, 2020a, 2020b). In contrast, the results obtained in this study indicate that foliar fertilizer did not increase SPAD index that would indicate more abundant chlorophyll amount. At this stage of understanding it is believed that foliar fertilizer slightly increased photosynthetic activity in maize but, however, it did not show a significant extent.

For the accurate determination of nutrient supply of maize leaves, a laboratory leaf analysis was performed on sampled adult leaves. Results are presented in Table 2. Leaf analysis supports the idea that maize that was not treated with N-stabilizer (C; F) showed N-content below the critical value (critical value: N: 28,000 mg kg^{-1}; Elek and Kádár, 1980), which was also indicated by the typical N-deficiency symptoms (yellowing of aging leaves; Bergmann, 1992; Voss, 1993). On the other hand, nitrapyrin treated maize (N; N+F) did not show any symptoms referring to N-deficiency and it was confirmed by the laboratory leaf analysis. It follows from the foregoing that nitrapyrin treatment contributed to prevent N-deficiency in maize and improve N-supply. This explains why the highest SPAD readings were detected in nitrapyrin treated maize.

In terms of maize sensitivity, particular attention was paid to the Zn and Mg supply (Gupta et al., 2008; Mengutay et al., 2013). Results indicate that for both nutrients hidden nutrient deficiency developed in control (C) and treated (N; F; N+F) maize as both nutrients were below the critical minimum value (critical minimum value: Mg: 2,500 mg kg^{-1}; Zn: 25 mg kg^{-1}; Elek and Kádár, 1980) despite no visual symptoms.

Table 2. The total nutrient content of maize leaves due to each treatment

Treatments	C	N	F	N+F
Nitrogen (N)	23,900 ± 1,195[a]	28,100 ± 1,405	26,300 ± 1,315[a]	29,100 ± 1,455
Phosphorus (P)	2,170 ± 87	2,510 ± 100	2,210 ± 88	2,550 ± 102
Potassium (K)	17,700 ± 708	17,100 ± 684	16,630 ± 665	17,070 ± 683
Calcium (Ca)	4,230 ± 317	4,220 ± 317	3,990 ± 299	4,300 ± 323
Magnesium (Mg)	1,560 ± 117[b]	1,650 ± 124[b]	1,500 ± 113[b]	1,620 ± 122[b]
Sulphur (S)	1,480 ± 148	1,840 ± 184	1,590 ± 159	1,910 ± 191
Boron (B)	10 ± 1.3	11 ± 1.4	18 ± 2.3	14 ± 1.8
Copper (Cu)	5.6 ± 0.4	7.4 ± 0.6	6.1 ± 0.5	8.1 ± 0.6
Iron (Fe)	92.8 ± 7.0	115.0 ± 5.8	135.0 ± 6.8	121.0 ± 6.1
Manganese (Mn)	47.7 ± 3.6	53.5 ± 4.0	48.1 ± 3.6	57.6 ± 4.3
Zinc (Zn)	10.4 ± 0.8[c]	14.5 ± 1.1[c]	18.3 ± 1.4[c]	14.1 ± 1.1[c]

All values in mg per kg of air-dried substance; [a] - value less than the lower critical value of the optimal content (28,000 mg kg^{-1}; Elek and Kádár, 1980); [b] - value less than the lower critical value of optimal content (25,00 mg kg^{-1}; Elek and Kádár, 1980); [c] - value less than the lower critical value of optimal content (25 mg kg^{-1}; Elek and Kádár, 1980); C: untreated control; N: nitrapyrin; F: foliar fertilizer; N+F: nitrapyrin and foliar fertilizer

This can be attributed to the fact that the nutrient composition of the applied foliar fertilizer was general, which did not include Mg, and contained only a small amount (38 mg L^{-1}) of Zn. Therefore, it remains unclear whether the development of deficiency disease could have been avoided if the foliar fertilizer had contained Mg and more Zn. Teklic et al. (2009) and Júnior et al. (2019) have reported that Mg fertilized plants might cause increase in photosynthetic activity, chlorophyll values, and SPAD index. It can be explained by the fact that Mg is a component of the chlorophyll molecule, thus, it has a central role in the utilization of light energy in photosynthesis. As a result, adequate Mg nutrition is essential in crop production especially under stress conditions (Cakmak and Kirkby, 2008; Verbruggen and Hermans, 2013).

Several previous studies have reported increasing sensitivity of maize to Zn deficiency (Wang and Jin, 2007; Gupta et al., 2008; Mattiello et al., 2015), which provides an explanation for the Zn-deficiency developed in all treated maize in this research and this may be the reason why low-Zn foliar fertilizer is not satisfactory to cover nutritional requirements of maize. However, it needs to be emphasized that despite the low-Zn content foliar fertilizer, it was still able to improve Zn-supply compared to untreated maize. An important implication of these findings is that due to increased sensitivity of maize to Zn and Mg-deficiency, these nutrients are particularly important to be applied with foliar fertilizers designed in the nutrient needs of maize.

Tolerance to maize-specific pathogens was tested by artificial maize ear infection with *F. verticillioides* (*Fv*) and *A. flavus* (*Af*) filamentous fungi. Degrees of infections are represented in Fig. 3 which suggests that both pathogens were more infective in untreated (C) crops (*Fv*: 3.86 ± 0.70%; Af: 3.14 ± 0.45%), while the infection was the least intense in maize treated with nitrapyrin and foliar fertilizer (N+F; *Fv*: 0.26 ± 0.45%; *Af*: 0.46 ± 0.33%). However, there was no significant difference between the infection symptoms observed in the combined treatment (N+F) and treatment with foliar fertilizer (F) alone and the intensity of symptoms caused by *F. verticillioides* in nitrapyrin treatment

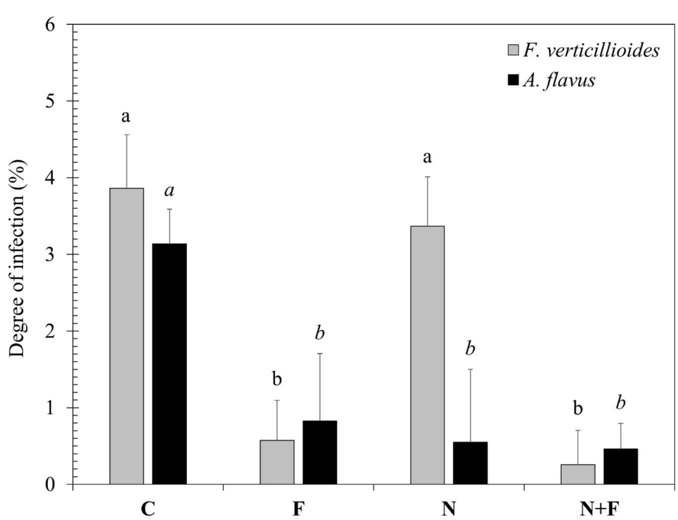

Figure 2. The intensity of maize ear infection caused by *F. verticillioides* and *A. flavus* due to each treatment. C: untreated control; F: foliar fertilizer; N: nitrapyrin; N+F: nitrapyrin and foliar fertilizer; Different lowercase letters denote statistically significant differences (Duncan's multiple comparison test, P ≤ 0.05) between treatments

(N) maize was similar to the untreated, control (C) maize. We speculate that this might be due to the more abundant N in the soil utilized by maize that was provided by nitrapyrin, which is in line with the findings by Reid et al. (2001) providing evidence for increasing disease severity due to higher rates of N. Overall, results obtained in current study suggest that maize with poor nutrient supply induced the lowest tolerance to maize ear infection, which is in accordance with thee fact that optimal nutrient supply plays a key role to decreased disease severity (Dordas, 2008; Gupta et al., 2017). Although the findings support the beneficial effect of foliar fertilizer treatment, it is believed that its efficiency would have been enhanced further if it had contained higher Zn and Mg content instead of general composition of nutrients.

Conclusions

As an outcome of the examinations, the application of nitrapyrin can be recommended at the same time as post-emergent herbicide treatment to enhance N-use efficiency as in this way adequate amount of available N can be ensured in the soil during the high N-demanding period of maize. Contrary to initial expectations, foliar fertilizer treatment slightly improved the maize nutrient supply as the amount of Mg and Zn was still critical in treated crops. In the opinion of the authors, this may have been the result of the general nutrient composition and low Zn content of the applied foliar fertilizer.

The tolerance to the artificial ear infection by the two pathogens was improved mostly due to the combination of two technologies (N-stabilizer and foliar fertilizer). Although the results suggest that foliar fertilized maize showed significantly reduced symptoms of the infection, it is believed that the beneficial effect of foliar fertilizer could have been enhanced further by a special product designed to the individual nutrient supply of maize. Results draw the attention to the reasonable N-supply as well as optimal Zn and Mg supply due to the increased sensitivity to deficiencies of maize.

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