Effect of nitrification inhibitors on the content of available nitrogen forms in the soil under maize (*Zea mays*, L.) growing

Vplyv inhibítorov nitrifikácie na obsah prijateľných foriem pôdneho dusíka pri pestovaní kukurice siatej (*Zea mays*, L.)

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Abstract

The objective of this research was to investigate the effect of nitrification inhibitors (dicyandiamide and 1,2,4 triazole) on the content of nitrate and ammonium nitrogen in the soil and the effectiveness of nitrogen-sulphur nutrition of maize. The research was conducted in field small-plot experiment with maize on Haplic Luvisol with dominance of clay fraction in experimental years 2012 to 2015. The dose of nitrogen in all experimental treatments was 160 kg*ha^{-1} and was applied at one shot or split in three partial doses. Soil samples from all examined treatments were taken from three soil depths (0.0-0.3 m, 0.3-0.6 m and 0.6-0.9 m, respectively) by probe rod in 4-5 week intervals. Achieved results indicate that on the average of four years and three depths of the soil profile, application of nitrification inhibitors contained in fertilizer ENSIN considerably reduced portion of nitrate nitrogen from the content of mineral nitrogen in the soil by 7-32 relative %. The application of fertilizer ENSIN considerably increased content of ammonium nitrogen in the soil by 10-59 relative %. A favourable effect on increase of ammonium nitrogen content and reduction of nitrate nitrogen content was found out in spite of the fact that in this treatment the total dose of fertilizer was applied at one shot.

Keywords: ammonium cation, fertilization, nitrates, nitrification, nitrification inhibitors

Abstrakt

V pestovateľských rokoch 2012, 2013, 2014 a 2015 bol založený maloparcelkový poľný pokus s pestovaním kukurice siatej na stredne ťažkej hnedozemi s prevahou ílových častíc na lokalite Horné Semerovce. Pred sejbou kukurice sa z pôdneho profilu 0,0-0,3 m a 0,3-0,6 m odobrali pôdne vzorky k agrochemickým analýzam. Cieľom výskumu bolo skúmanie vplyvu inhibítorov nitrifikácie (dikyándiamid a 1,2,4 triazol) na obsah dusičnanového a amónneho dusíka v pôde a efektívnosti dusíkato-sírnej výživy kukurice siatej. Dávka čistého dusíka vo všetkých pokusných variantoch bola 160 kg*ha^{-1}. V poľnom pokuse boli použité nasledovné dusíkaté hnojivá: liadok amónny s dolomitom LAD 27 (27% N), dusičnan amónny + síran amónny DASA 26/13 (26% N, 13% S) a ENSIN s rovnakým obsahom živín ako hnojivo DASA 26/13, obsahujúci dva inhibítory nitrifikácie. Inhibítory nitrifikácie dikyándiamid (DCD) a 1,2,4 triazol (TZ) sú priamo začlenené do granúl hnojiva ENSIN a tvoria jeho neoddeliteľnú súčasť. Hnojivá LAD 27 a DASA 26/13 boli aplikované 3-krát delenou aplikáciou pred sejbou kukurice, počas sejby kukurice a pri výške rastlín kukurice 0,3 m. Hnojivo ENSIN bolo aplikované jednorazovo pred sejbou kukurice. Každý variant bol štvornásobne opakovaný a plocha jedného opakovania bola 100 m^2 (20 m x 5 m). V pokuse bolo 16 parceliek o celkovej výmere pokusu 1 600 m^2 (4 varianty x 4 opakovania = 16 parceliek x 100 m^2 = 1 600 m^2). Pôdne vzorky zo všetkých skúmaných variantov sa odoberali z pôdneho profilu 0,0-0,3 m, 0,3-0,6 m a 0,6-0,9 m. Počas vegetácie kukurice sa uskutočnilo 6 odberov pôdnych vzoriek v 4-5 týždenných intervaloch až do zberu kukurice v pestovateľských rokoch 2012-2014. V pestovateľskom roku 2015 sa uskutočnilo 5 odberov pôdnych vzoriek počas vegetácie kukurice. Najnižšie hodnoty amónneho ($N\text{-}NH_4^+$), dusičnanového ($N\text{-}NO_3^-$) a anorganického dusíka (N_{an}) boli na kontrolnom nehnojenom variante vo všetkých pokusných rokoch. Relatívne vyššie obsahy dusičnanového dusíka oproti amónnemu dusíku v pôde sa nachádzali pri aplikovaní hnojív LAD 27 a DASA 26/13. Pri aplikovaní hnojiva ENSIN sa prejavila inhibícia nitrifikácie postupným uvoľňovaním amónneho dusíka, čo spôsobilo vyššie obsahy amónneho dusíka ako dusičnanového dusíka hlavne v mesiacoch máj až júl. Najvyššie obsahy amónneho dusíka a zároveň aj obsahy anorganického dusíka počas celej vegetácie boli na variante s hnojivom ENSIN. Dosiahnuté výsledky ukazujú, že v priemere štyroch pokusných rokov a troch hĺbok pôdneho profilu, aplikácia inhibítorov nitrifikácie v hnojive ENSIN výrazne znížila podiel dusičnanového dusíka z obsahu anorganického dusíka v pôde o 7-32 relatívnych %. Aplikácia hnojiva ENSIN výrazne zvýšila obsah amónneho dusíka v pôde o 10-59 relatívnych %. Priaznivý vplyv na zvyšovanie obsahu amónneho dusíka a na znižovanie dusičnanového dusíka v pôde bol zistený, aj napriek skutočnosti, že celková dávka hnojiva ENSIN bola aplikovaná jednorazovo pred sejbou kukurice.

Kľúčové slová: amónny katión, dusičnany, hnojenie, inhibítory nitrifikácie, nitrifikácia

Introduction

Agricultural intensification has led to high inputs of nitrogen fertilizer into cultivated land (Qu et al., 2014). One of the most important factors affecting crop yields is nitrogen fertilization (Ambus et al., 2011; Kajanovičová et al., 2011). Excessive application of nitrogen, however, results in low use efficiency and high nitrogen losses, and causes environmental problems (Gentile et al., 2009; Sutton et al., 2011; Liu et al., 2015).

Nitrate is a highly labile and mobile nitrogen form that can be lost from agricultural systems through leaching and runoff, and released in gaseous forms (N_2O and NO through denitrification) (Upadhyay et al., 2011). Nitrous oxide is produced in the soil mainly by two biological processes nitrification and denitrification (Ambus et al., 2006; Aita et al., 2015; Barneze et al., 2015). Nitrification, a microbial process, is a key component and integral part of the soil nitrogen cycle. Nitrification may enhance the loss of fertilizer nitrogen by $N\text{-}NO_3^-$ leaching and denitrification (Gong et al., 2013). $N\text{-}NO_3^-$ leaching from agricultural soils is one of the crucial global environmental problems. These losses contribute to nitrate contamination of surface and groundwater.

One of the mitigation technologies that have proved to be highly effective in reducing fertilizer nitrogen losses and increasing nitrogen use efficiency in new cropping systems is the application of nitrification inhibitors (Cui et al., 2011; Moir et al., 2012). Nitrification inhibitors are chemical compounds which are able to delay the stepwise microbial oxidation of ammonium via nitrite to nitrate (Kleineidam et al., 2011; Qiao et al., 2015). Dicyandiamide is one type of highly effective nitrification inhibitor (Liu et al., 2015). Dicyandiamide applied at 10 $kg*ha^{-1}$ had been used as effective management tool for blocking nitrification (Di et al., 2007; Zaman et al., 2007; Zaman et al., 2009). Lower soil NO_3^- concentration resulting from the inhibition of nitrification also reduces substrate availability for denitrification (Akiyama et al., 2010). The extent to which dicyandiamide inhibits N_2O emission and NO_3^- leaching is primarily dependent on factors such as the application rate, time and method of nitrification inhibitor application (Zaman and Blennerhassett, 2010; Zaman and Nguyen, 2012). Other factors include field management (Sanz-Cobena et al., 2012), climate (precipitation and temperature) and soil properties (Shepherd et al., 2012). Triazole compounds constitute an important class of organic chemistry due to their various biological and corrosion inhibition activities (Srivastava et al., 2016).

Developing management strategies and tools for reducing greenhouse gas emissions and minimizing nitrogen loss from soils is crucial. Adopting mitigation options at large scale by farmers requires simple and effective tools, as has been the case for nitrification inhibitors.

Within this context, the aim of this study was to determine the effect of nitrification inhibitors on the content of nitrate and ammonium nitrogen in the soil and the effectiveness of nitrogen-sulphur fertilization in maize.

Materials and methods

Four-year small-plot experiment with maize growing was established on Haplic Luvisol with dominance of clay fraction in locality of Horné Semerovce, Farm Agrosemeg S3, s.r.o. (E 48°13´; N 18°88´) in the years 2012 to 2015. Current temperatures and precipitations in respective experimental years, as well as long term normal are stated in Table 1 and 2. Soil samples for agrochemical analyses were taken before seeding of maize from soil depth of 0.0 to 0.3 m and 0.3 to 0.6 m, respectively. Agrochemical characteristics of soil samples and their evaluations are stated in Table 3 and 4. In respective years seeding of maize was carried out at the end of April and the harvest was performed in the first decade of October.

Table 1. Review of average temperatures in locality of Horné Semerovce (°C)

Tabuľka 1. Prehľad priemerných teplôt na lokalite Horné Semerovce (°C)

Month	2012	2013	2014	2015	30-year normal	difference 2012	difference 2013	difference 2014	difference 2015
January	1.4	-1.1	2.7	1.4	-2.1	+3.5	+1.0	+4.8	+3.5
February	-3.0	1.5	4.6	1.8	0.0	-3.0	+1.5	+4.6	+1.8
March	7.5	3.5	8.9	6.6	5.0	+2.5	-1.5	+3.9	+1.6
April	12.9	13.0	13.0	11.2	9.6	+3.3	+3.4	+3.4	+1.6
May	17.6	17.2	16.4	16.9	15.1	+2.5	+2.1	+1.3	+1.8
June	21.3	20.7	21.5	21.7	17.9	+3.4	+2.8	+3.6	+3.8
July	23.1	24.6	23.5	25.2	19.9	+3.2	+4.7	+3.6	+5.3
August	22.2	23.7	19.8	24.8	19.3	+2.9	+4.4	+0.5	+5.5
September	17.6	14.8	17.2	18.0	14.7	+2.9	+0.1	+2.5	+3.3

Table 2. Review of precipitation in locality of Horné Semerovce (mm)

Tabuľka 2. Prehľad zrážok na lokalite Horné Semerovce (mm)

Month	2012	2013	2014	2015	30-year normal	difference 2012	difference 2013	difference 2014	difference 2015
January	34	83	37	31	41	-7.0	+42.0	-4.0	-10.0
February	37.5	66	56	5	30	+7.5	+33.0	+26.0	-25.0
March	0	93	21	16	37	-37.0	+56.0	-16.0	-21.0
April	46	24	37	20	39	+7.0	-15.0	-2.0	-19.0
May	15	145	84	45	59	-44.0	+86.0	+25.0	-14.0
June	31	116	35	13	68	-37.0	+48.0	-33.0	-55.0
July	86	16	89	43	48	+38.0	-32.0	+41.0	-5.0
August	0	55	124	59	47	-47.0	+8.0	+77.0	+12.0
September	35	55	79	63	42	-7.0	+13.0	+37.0	+21.0
October	80.5	-	22	-	40	+40.5	-	-18.0	-
Apr.-Sept.	213	411	448	243	303	-90.0	+108.0	+145.0	-60.0

Table 3. Agrochemical characteristic of soil in locality of Horné Semerovce (0.0-0.3 m)

Tabuľka 3. Agrochemická charakteristika pôdy na lokalite Horné Semerovce (0,0-0,3 m)

Year	mg*kg^{-1}						Humus	pH/KCl
	N_{min}	P	K	Ca	Mg	S	%	
2012	29.9D	42.5N	301V	2300S	301V	12.5N	2.73S	5.78
2013	29.4D	160V	385V	2780S	439VV	27.5S	3.54D	6.92
2014	30.4D	45N	201D	3580D	431VV	1.9VN	3.09D	6.16
2015	28.5D	41.3N	483VV	2350S	589VV	4.4VN	2.68S	5.32

VN - very low content, N - low content, S - medium content, D - good content, V - high content, VV - very high content

VN - veľmi nízky obsah, N - nízky obsah, S - stredný obsah, D - dobrý obsah, V - vysoký obsah, VV - veľmi vysoký obsah

Table 4. Agrochemical characteristic of soil in locality of Horné Semerovce (0.3-0.6 m)

Tabuľka 4. Agrochemická charakteristika pôdy na lokalite Horné Semerovce (0,3-0,6 m)

Year	mg*kg^{-1}						Humus	pH/KCl
	N_{min}	P	K	Ca	Mg	S	%	
2012	16.2S	22.5VN	200S	2190S	295V	12.5N	1.75N	6.23
2013	14.2S	148V	290D	2280S	413VV	10.6N	2.38S	6.91
2014	8.8N	22.5VN	140S	3850D	493VV	5VN	2.88S	6.24
2015	16.2S	16.3VN	250D	2880S	665VV	1.3VN	2.39S	5.69

VN - very low content, N - low content, S - medium content, D - good content, V - high content, VV - very high content

VN - veľmi nízky obsah, N - nízky obsah, S - stredný obsah, D - dobrý obsah, V - vysoký obsah, VV - veľmi vysoký obsah

The scheme of maize fertilization treatments and the doses of nitrogen applied per hectare at respective growth stages are stated in Table 5. The following fertilizers were applied in the experiment: nitrogen fertilizer Ammonium nitrate with dolomite LAD 27 (27% N), nitrogen-sulphur fertilizer Ammonium nitrate + Ammonium sulphate DASA 26/13 (26% N, 13% S) and fertilizer ENSIN (with the same content of nutrients as fertilizer DASA 26/13) which contains two nitrification inhibitors. Nitrification inhibitors of interest were represented by dicyandiamide (DCD) and 1,2,4 triazole (TZ) which were incorporated directly in the granule of fertilizer ENSIN as its integral part.

Table 5. Scheme of maize fertilization treatments

Tabuľka 5. Schéma variantov výživy kukurice siatej

Nutrition treatment	Fertilizer	Before maize seeding		At maize seeding		Maize height of 0.3 m	
		Doses of nutrients (kg*ha^{-1})					
		N	S	N	S	N	S
1	control, without fertilization	-	-	-	-	-	-
2	LAD 27	60	-	60	-	40	-
3	DASA 26/13	60	30	60	30	40	20
4	ENSIN	160	80	-	-	-	-

Fertilizers LAD 27 and DASA 26/13 were applied three times by split application before seeding of maize, during seeding of maize and at the height of maize of 0.3 m, respectively. Fertilizer ENSIN was applied by only one shot application before seeding of maize.

Each treatment was replicated four times and each plot was represented by the area of 100 m^2 (20 m x 5 m). The maize vegetation was treated against weeds, diseases and pests by use of standard methods of agrochemical application.

In all treatments, soil samples were taken from soil depth of 0.0 to 0.3 m, 0.3 to 0.6 m and 0.6 to 0.9 m in the year 2012 and depth of 0.0 to 0.3 m and 0.3 to 0.6 m in the years 2013 to 2015. There were 6 samplings conducted in an interval of 4 to 5 weeks during maize growing season in the years 2012 to 2014. In the year 2015, there were 5 samplings taken during maize growing season.

The contents of available phosphorus and potassium (in leachate of Mehlich III) were determined by colorimetry and by the method of flame photometry, respectively. The contents of available calcium and magnesium were measured using the atomic absorption spectrophotometer. The content of available sulphur was determined in the solution of ammonium acetate by colorimetry. The exchange soil acidity (pH_{KCl}) was set in the leachate of 0.2 M potassium chloride (KCl). The content of soil humus was set by the method of Tjurina in taken soil samples before seeding maize. The contents of nitrate and ammonium nitrogen (in 1% leachate of K_2SO_4) were determined by the colour method with phenol 2.4-disulfonic acid and by the colour method with Nessler test solution in taken soil samples before seeding maize and during maize growing season, respectively. The content of mineral nitrogen (N_{min}) in the soil was calculated as the sum of ammonium (N-NH_4^+) and nitrate (N-NO_3^-) nitrogen content. Nitrate and ammonium nitrogen portions from the content of mineral nitrogen in the soil were statistically evaluated by analysis of variance and the differences between treatments were assessed by Tukey test.

Results

Air temperature was higher than a long term normal by 2.5 to 3.6 °C during the maize growing season in all four experimental years (Table 1). However, considerable differences were found in both amount and distribution of atmospheric precipitation influencing the final contents of nitrate (N-NO_3^-) and ammonium (N-NH_4^+) nitrogen in the soil (Table 2).

The lowest values of nitrate nitrogen ($N\text{-}NO_3^-$), ammonium nitrogen ($N\text{-}NH_4^+$) and mineral nitrogen (N_{min}) were observed at control treatment without nitrogen fertilization in all four experimental years. The contents of $N\text{-}NO_3^-$, $N\text{-}NH_4^+$, as well as N_{min} in the soil were ranged at higher levels, as a result of higher application doses of nitrogen (160 $kg*ha^{-1}$). Relatively higher contents of nitrate nitrogen in the soil were found after the application of fertilizers LAD 27 and DASA 26/13 during maize growing season, compared to contents of ammonium nitrogen.

The soil $N\text{-}NH_4^+$ contents ranged from 7 to 14 $mg*kg^{-1}$ soil in the year 2012 (Table 6). The content of nitrification inhibitors in fertilizer ENSIN slowed down the intensity of nitrate leaching. The highest contents of ammonium nitrogen, as well as mineral nitrogen, were found at treatment fertilized with ENSIN which contains nitrification inhibitors. The contents of nitrate nitrogen were almost the same at all fertilized treatments and ranged from 16 to 17 $mg*kg^{-1}$ soil. The effect of nitrification inhibitors was reflected at treatment fertilized with ENSIN, and consequently the portion of $N\text{-}NO_3^-$ from N_{min} was reduced by 7 relative % and portion of ammonium nitrogen was increased by 10 relative % in comparison with treatment fertilized with DASA 26/13. Even greater inhibitory effect was found at treatment fertilized with ENSIN in comparison with treatment, where fertilizer LAD 27 was applied (Figure 1).

Table 6. Content of ammonium and nitrate nitrogen ($mg*kg^{-1}$ soil) and portion of nitrogen forms from N_{min} in the soil (%) in 2012 (average of sampling dates and replications)

Tabuľka 6. Obsah amónneho a dusičnanového dusíka ($mg*kg^{-1}$ pôdy) a podiel dusíkových foriem z obsahu N_{an} v pôde (%) v roku 2012 (priemer odberov a opakovaní)

Nutrition treatment	Content of N ($mg*kg^{-1}$ soil)		N_{min} ($mg*kg^{-1}$ soil)	Portion of N from N_{min} in the soil (%)	
	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$		$N\text{-}NH_4^+$	$N\text{-}NO_3^-$
Control	7	9	16	44	56
LAD 27	11	17	28	39	61
DASA 26/13	11	16	27	41	59
ENSIN	14	17	31	45	55

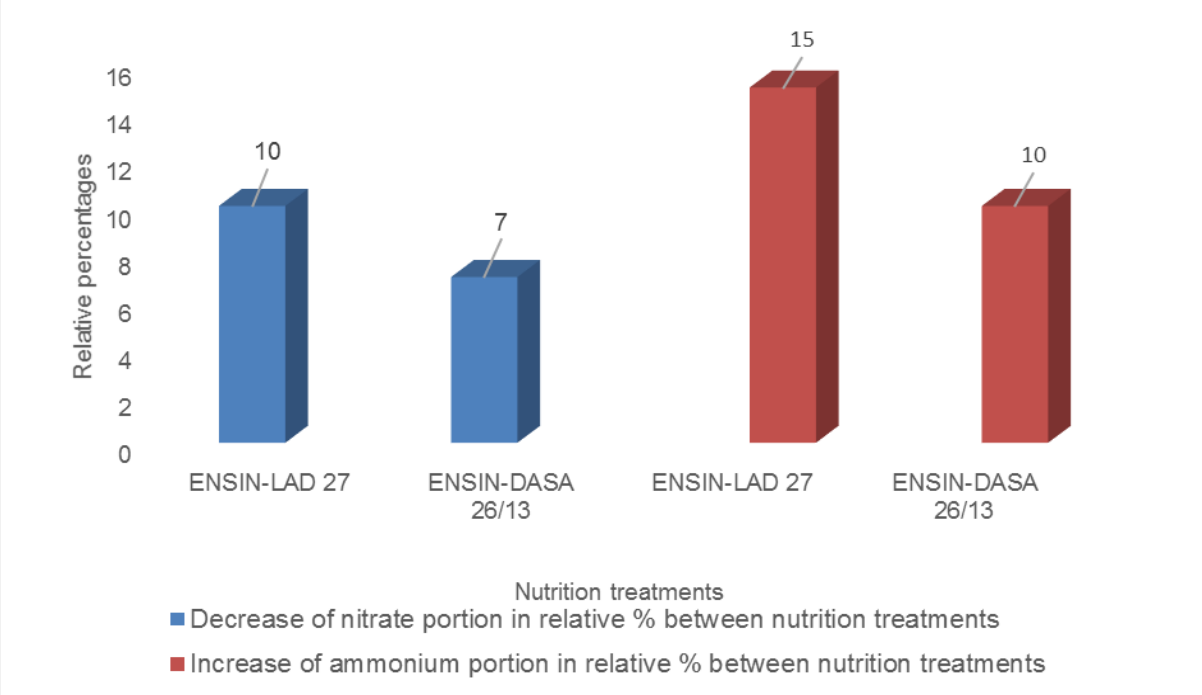

Figure 1. Decrease of nitrate portion at treatment ENSIN compared to treatments LAD 27 and DASA 26/13 and increase of ammonium portion at treatment ENSIN compared to treatments LAD 27 and DASA 26/13 in relative % in 2012

Obrázok 1. Zníženie podielu dusičnanov na variante ENSIN v porovnaní s variantmi LAD 27 a DASA 26/13 a zvýšenie podielu amónneho dusíka na variante ENSIN v porovnaní s variantmi LAD 27 a DASA 26/13 v relatívnych % v roku 2012

In 2013, the contents of mineral nitrogen ranged from 16 to 30 mg*kg^{-1} soil (Table 7). The highest and simultaneously the same values of N_{min} were determined at treatments fertilized with LAD 27 and DASA 26/13 (30 mg*kg^{-1}). The same contents of N-NO_3^- were found at treatments fertilized with LAD 27 and DASA 26/13 (20 mg*kg^{-1}). The highest amount of N-NH_4^+ was found at treatment fertilized with ENSIN (11 mg*kg^{-1}). Nitrification inhibitors which formed part of nitrogen-sulphur fertilizer ENSIN had positive effect on reduction of nitrates in the soil. The soil N-NO_3^- content was decreased by 9 relative % at treatment fertilized with ENSIN, in comparison with treatments, where fertilizers LAD 27 and DASA 26/13 without inhibitors of nitrification were applied (Figure 2). The soil N-NH_4^+ content was increased by 18 relative % at treatment fertilized with ENSIN, in comparison with treatments fertilized with fertilizers without inhibitors of nitrification.

Table 7. Content of ammonium and nitrate nitrogen (mg*kg^{-1} soil) and portion of nitrogen forms from N_{min} in the soil (%) in 2013 (average of sampling dates and replications)

Tabuľka 7. Obsah amónneho a dusičnanového dusíka (mg*kg^{-1} pôdy) a podiel dusíkových foriem z obsahu N_{an} v pôde (%) v roku 2013 (priemer odberov a opakovaní)

Nutrition treatment	Content of N (mg*kg^{-1} soil)		N_{min} (mg*kg^{-1} soil)	Portion of N from N_{min} in the soil (%)	
	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$		$N\text{-}NH_4^+$	$N\text{-}NO_3^-$
Control	7	9	16	44	56
LAD 27	10	20	30	33	67
DASA 26/13	10	20	30	33	67
ENSIN	11	17	28	39	61

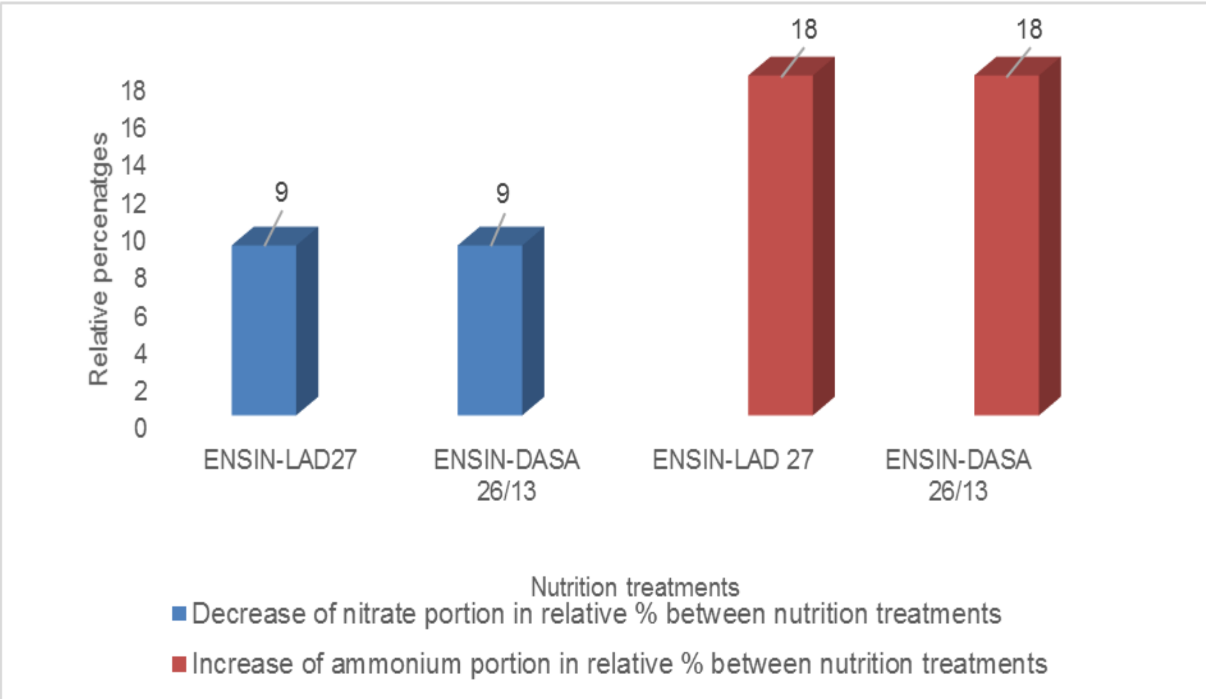

Figure 2. Decrease of nitrate portion at treatment ENSIN compared to treatments LAD 27 and DASA 26/13 and increase of ammonium portion at treatment ENSIN compared to treatments LAD 27 and DASA 26/13 in relative % in 2013

Obrázok 2. Zníženie podielu dusičnanov na variante ENSIN v porovnaní s variantmi LAD 27 a DASA 26/13 a zvýšenie podielu amónneho dusíka na variante ENSIN v porovnaní s variantmi LAD 27 a DASA 26/13 v relatívnych % v roku 2013

The application of nitrification inhibitors considerably decreased the soil $N\text{-}NO_3^-$ content and greatly increased the soil $N\text{-}NH_4^+$ content. The highest concentrations of N_{min} (41 mg*kg^{-1}) and $N\text{-}NH_4^+$ (21 mg*kg^{-1}) were found at fertilized treatment, where fertilizer ENSIN with two nitrification inhibitors was applied in the experimental year 2014 (Table 8). The soil N-NO_3^- concentrations ranged from 8 to 25 mg*kg^{-1} soil. At treatment fertilized with LAD 27, there was the highest content of N-NO_3^- (25 mg*kg^{-1}) observed. The most considerable effect of nitrification inhibitors was determined at application of fertilizer ENSIN. Nitrification inhibitors dicyandiamide and 1,2,4 triazole limited the nitrification process. This resulted in a reduction of soil N-NO_3^- content by 28 relative % and the considerable increase of N-NH_4^+ content by 59 relative % compared to treatment fertilized with LAD 27. A slight decrease of N-NO_3^- content and increase of N-NH_4^+ content by 14 relative % and 19 relative %, respectively was found in the plots treated with fertilizer ENSIN in comparison with the plots, where fertilizer DASA 26/13 was applied (Figure 3).

Table 8. Content of ammonium and nitrate nitrogen (mg*kg^{-1} soil) and portion of nitrogen forms from N_{min} in the soil (%) in 2014 (average of sampling dates and replications)

Tabuľka 8. Obsah amónneho a dusičnanového dusíka (mg*kg^{-1} pôdy) a podiel dusíkových foriem z obsahu N_{an} v pôde (%) v roku 2014 (priemer odberov a opakovaní)

Nutrition treatment	Content of N (mg*kg^{-1} soil)		N_{min} (mg*kg^{-1} soil)	Portion of N from N_{min} in the soil (%)	
	N-NH_4^+	N-NO_3^-		N-NH_4^+	N-NO_3^-
Control	7	8	15	47	53
LAD 27	12	25	37	32	68
DASA 26/13	16	21	37	43	57
ENSIN	21	20	41	51	49

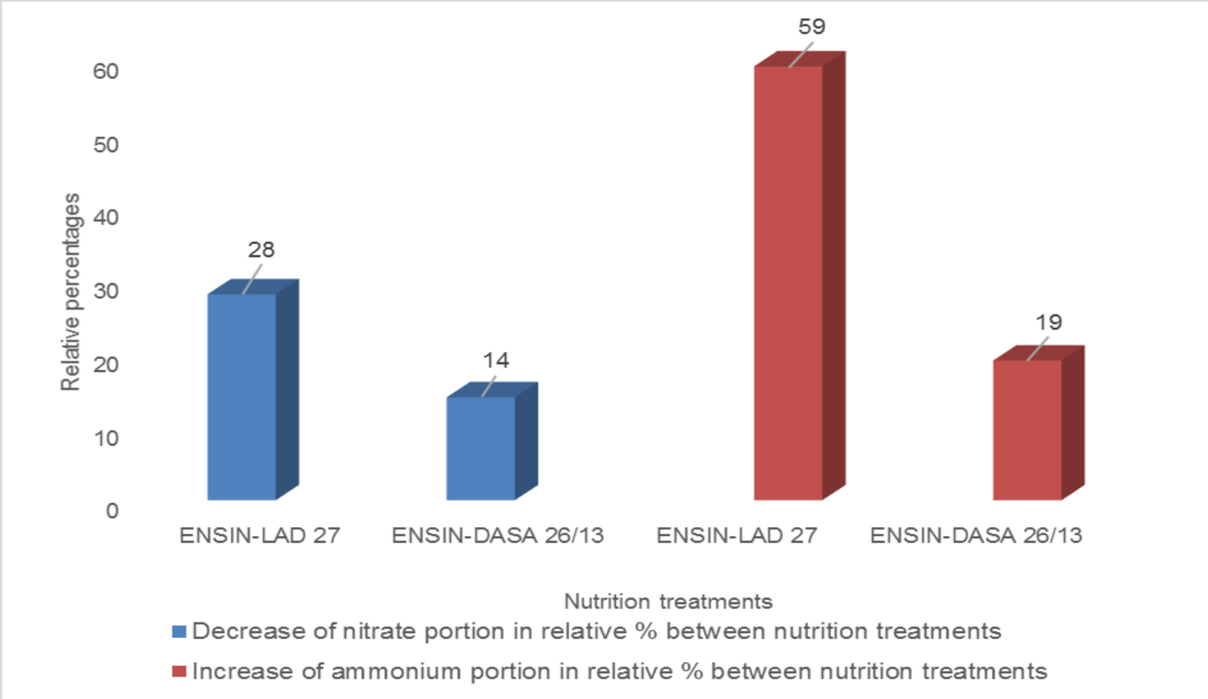

Figure 3. Decrease of nitrate portion at treatment ENSIN compared to treatments LAD 27 and DASA 26/13 and increase of ammonium portion at treatment ENSIN compared to treatments LAD 27 and DASA 26/13 in relative % in 2014

Obrázok 3. Zníženie podielu dusičnanov na variante ENSIN v porovnaní s variantmi LAD 27 a DASA 26/13 a zvýšenie podielu amónneho dusíka na variante ENSIN v porovnaní s variantmi LAD 27 a DASA 26/13 v relatívnych % v roku 2014

Addition of nitrification inhibitors dicyandiamide and 1,2,4 triazole to fertilizer ENSIN maintained soil nitrogen in ammonium form $N\text{-}NH_4^+$. The highest values of N_{min} (35 $mg*kg^{-1}$) and $N\text{-}NH_4^+$ (21 $mg*kg^{-1}$) were observed at treatment fertilized with nitrogen-sulphur fertilizer ENSIN with nitrification inhibitors in the experimental year 2015 (Table 9). The conversion processes between $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ (nitrification and denitrification) were inhibited by the nitrification inhibitors. Substantially lower concentration of soil $N\text{-}NO_3^-$ was observed in the plots treated with fertilizer ENSIN containing two inhibitors. Nitrification inhibitors lower nitrate leaching and nitrous oxide emission by preventing or slowing the microbial conversion of ammonium nitrogen to nitrate nitrogen (Lam et al., 2015). Comparatively the highest contents of $N\text{-}NO_3^-$ were found after the application of fertilizers LAD 27 and DASA 26/13. The most substantial effect of nitrification inhibitors was reflected in this experimental year, in comparison with other experimental years. Reduction of $N\text{-}NO_3^-$ portion was about 32 relative % at treatment fertilized with ENSIN, in comparison with fertilized treatments, where fertilizers LAD 27 and DASA 26/13 were applied (Figure 4). Considerably higher content of soil $N\text{-}NH_4^+$ was observed in the plots treated with fertilizer ENSIN containing two inhibitors, where increase of $N\text{-}NH_4^+$ portion was about 46 relative %, in comparison with treatments fertilized with LAD 27 and DASA 26/13.

Table 9. Content of ammonium and nitrate nitrogen (mg*kg^{-1} soil) and portion of nitrogen forms from N_{min} in the soil (%) in 2015 (average of sampling dates and replications)

Tabuľka 9. Obsah amónneho a dusičnanového dusíka (mg*kg^{-1} pôdy) a podiel dusíkových foriem z obsahu N_{an} v pôde (%) v roku 2015 (priemer odberov a opakovaní)

Nutrition treatment	Content of N (mg*kg^{-1} soil)		N_{min} (mg*kg^{-1} soil)	Portion of N from N_{min} in the soil (%)	
	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$		$N\text{-}NH_4^+$	$N\text{-}NO_3^-$
Control	7	8	15	47	53
LAD 27	13	19	32	41	59
DASA 26/13	14	20	34	41	59
ENSIN	21	14	35	60	40

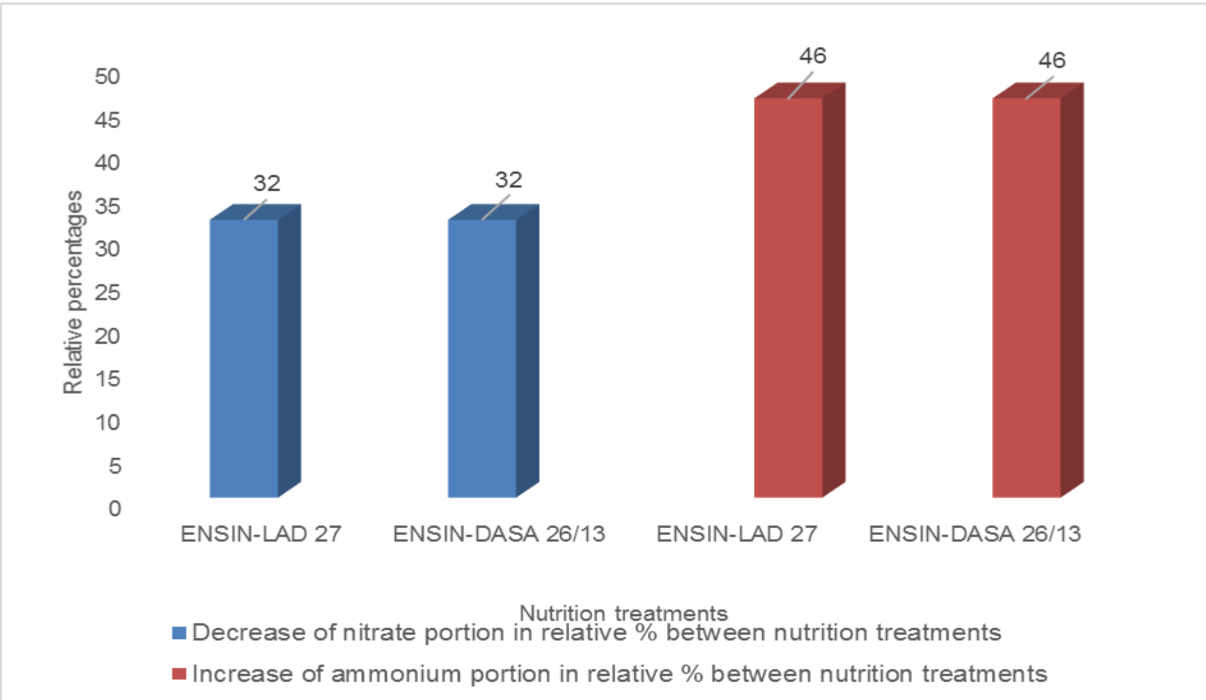

Figure 4. Decrease of nitrate portion at treatment ENSIN compared to treatments LAD 27 and DASA 26/13 and increase of ammonium portion at treatment ENSIN compared to treatments LAD 27 and DASA 26/13 in relative % in 2015

Obrázok 4. Zníženie podielu dusičnanov na variante ENSIN v porovnaní s variantmi LAD 27 a DASA 26/13 a zvýšenie podielu amónneho dusíka na variante ENSIN v porovnaní s variantmi LAD 27 a DASA 26/13 v relatívnych % v roku 2015

Table 10. Effect of nitrogen fertilization without addition of nitrification inhibitors and with addition of nitrification inhibitors on the content of ammonium nitrogen and nitrate nitrogen in the soil in 2012-2015

Tabuľka 10. Vplyv dusíkatého hnojenia bez pridania inhibítorov nitrifikácie a s pridaním inhibítorov nitrifikácie na obsah amónneho a dusičnanového dusíka v pôde v rokoch 2012-2015

Factor		Content of N ($mg*kg^{-1}$ soil)	
		$N\text{-}NH_4^+$	$N\text{-}NO_3^-$
Nutrition treatment			
Control		6.92^{aA}	8.54^{aA}
LAD 27		11.1^{bAB}	20.11^{bB}
DASA 26/13		12.38^{bBC}	18.83^{bB}
ENSIN		16.65^{cC}	17.24^{bB}
Experimental years			
2012		10.83^{abA}	14.7^{aA}
2013		9.35^{aA}	16.23^{aA}
2014		13.82^{bA}	18.58^{aA}
2015		13.06^{bA}	15.19^{aA}
LSD	0.05	3.48	4.27
treatment	0.01	4.61	5.66
LSD	0.05	3.41	4.18
year	0.01	4.52	5.54

α 0.05 - lowercase letters; α 0.01 - uppercase letters; The same letters at mean values represent statistically non-significant differences.

Statistically significant differences of contents of ammonium nitrogen in the soil were found between treatments without nitrogen fertilization (control) and treatments fertilized with nitrogen fertilizers (Table 10). Highly significant difference of $N\text{-}NH_4^+$ content was determined at treatment fertilized with ENSIN containing nitrification inhibitors, in comparison with fertilizers without nitrification inhibitors DASA 26/13 and LAD 27. Statistically non-significant differences of $N\text{-}NH_4^+$ contents were found between fertilizer LAD 27 and DASA 26/13. Statistically significant differences of contents of nitrate nitrogen in the soil were stated between treatments without nitrogen fertilization (control) and treatments fertilized with nitrogen fertilizers. Statistically non-significant differences of $N\text{-}NO_3^-$ contents were found at treatments fertilized with LAD 27, DASA 26/13 and ENSIN. The contents of ammonium nitrogen and nitrate nitrogen were not significantly affected by the experimental years.

Discussion

Nowadays an average 25% of plant-available nitrogen in soils (ammonium and nitrate) originates from the decomposition (mineralization) of organic nitrogen compounds in humus, plant and animal residues, and organic fertilizers, 5% from nitrogen in precipitations, and 70% from applied inorganic nitrogen fertilizers. Only about 50% of the applied nitrogen is taken up by corn during the year following fertilizer addition. About 25% is immobilized during residue decomposition or remains in the soil as nitrate. The remaining 25% is lost from the plant rooting zone by leaching and denitrification (Nelson and Huber, 2001). One approach is the use of nitrification inhibitors, which can effectively suppress conversion of N-NH_4^+ to N-NO_3^- in soil, and thus, application of nitrification inhibitors could reduce nitrate leaching, resulting in a reduction of surface and groundwater contamination. Dicyandiamide and triazole are two types of highly effective nitrification inhibitors. In this study, the nitrification inhibitors dicyandiamide and 1,2,4 triazole were efficient tools for reducing content of nitrate nitrogen and increasing content of ammonium nitrogen in the agricultural soil. Many authors (Cui et al., 2011; Di and Cameron, 2012; Moir et al., 2012; Pfab et al., 2012; Cahalan et al., 2015) state that, the application of dicyandiamide together with NH_4^+-based fertilizers has shown efficiency in reducing nitrogen losses in the form of NO_3^- leaching. Thus, a large number of studies have shown that nitrogen fertilizers with nitrification inhibitors are more environmentally friendly (Zaman et al., 2007; Zaman et al., 2009; Zaman and Blennerhassett, 2010; Ding et al., 2011). Gong et al. (2013) in the study observed that, without dicyandiamide (DCD) addition, soil content of N-NH_4^+ in treatment nitrogen declined sharply and was significantly lower than that in treatments N+DCD1 (ammonium sulfate + DCD1) and N+DCD2 (ammonium sulfate + DCD2) at day 7 and until the end of the experiment. Liu et al. (2013) observed that the application of dicyandiamide and 3,4-dimethylpyrazol phosphate slightly decreased the soil N-NO_3^- contents and significantly increased the soil N-NH_4^+ contents in the study. Malcolm et al. (2015) state that, the application of dicyandiamide reduced N-NO_3^- leaching losses by up to 54%. However, some previous studies have shown a shorter longevity and lower mobility of dicyandiamide compared with other nitrification inhibitors (Zerulla et al., 2001). The nitrification inhibitors suppress transformation of ammonium nitrate to nitrogen nitrate in the soil. During four experimental years, it was found out, that reduction of N-NO_3^- content ranged from 7 to 32 relative % and from 9 to 32 relative % at treatment fertilized with ENSIN containing inhibitors of nitrification dicyandiamide and 1,2,4 triazole, in comparison with nitrogen-sulphur fertilizer DASA 26/13 and nitrogen fertilizer LAD 27, respectively. The nitrification inhibitors contributed to gradual releasing of ammonium nitrogen, which resulted in higher concentrations of ammonium nitrogen compared to concentrations of nitrate nitrogen in the agricultural soil. The decrease in soil N-NO_3^- concentration under nitrification inhibitor addition resulted in a considerably decrease in N-NO_3^- leaching. The concentration of ammonium nitrogen in the soil increased under nitrification inhibitor application. In another study, it was determined that, the application of dicyandiamide reduced NO_3^- leaching by 22% (Kim et al., 2014). A series of studies reported by Di and Cameron over the past few years show a reduction of about 60 relative % in NO_3^- leaching (Di and Cameron, 2005; Di and Cameron, 2006; Di et al., 2007; Singh and Verma, 2007). Soil NH_4^+ and NO_3^- concentrations greatly increased after application of basal nitrogen fertilizers compared to the control (Ding et al., 2015). This citation is consistent with achieved results of ammonium and nitrate nitrogen contents in field experiment with maize growing in all experimental years 2012-2015. Nitrification inhibitors in the fertilizer ENSIN had positive effect on decrease of nitrates in the soil. It was observed that the application of fertilizer ENSIN containing two nitrification inhibitors dicyandiamide and 1,2,4 triazole decreased the soil nitrate nitrogen concentrations and substantially increased the soil ammonium nitrogen concentrations in all experimental years. It is important from the view point of environment, mainly protection of surface and groundwater against nitrate leaching. The advantages of usage of fertilizer ENSIN compared to fertilizers DASA 26/13 are that the fertilizer is applied in 1 dose and re-application of the fertilizer is not necessary. It allows farmers to save the time and costs, to increase the crop yields and allows better quality of cultivated crops. Indeed, a large number of studies have shown that the leaching of NO_3^- following the application of mineral nitrogen fertilizers can be significantly reduced by the addition of dicyandiamide (Ball-Coelho and Roy, 1999; Serna et al., 2000; Di and Cameron, 2002; Di and Cameron, 2012; Sanz-Cobena et al., 2012). In other study it was observed that, nitrate leaching from nitrification inhibitors treated plots were significantly less than relevant untreated plots. Statistically significant reduction in nitrate leaching loss was observed between soil treatments with and without nitrification inhibitors (Sivasakthy and Gnanavelrajah, 2012). Reduction of NO_3^- leaching loss was about 35-51% by the application of nitrification inhibitor (Fuchs and Schuster, 2008). Thus, the addition of nitrification inhibitors to the fertilizer ENSIN and other fertilizers contributes to protection of environment, mainly reduction of N-NO_3^- leaching into surface and groundwater and reduction of nitrogen losses to the atmosphere.

Conclusions

The nitrification inhibition was caused by application of the nitrogen-sulphur fertilizer ENSIN containing two nitrification inhibitors - dicyandiamide and 1,2,4 triazole. Inhibitors in the fertilizer ENSIN decreased the concentration of soil nitrate nitrogen and oppositely increased concentration of soil ammonium nitrogen. The nitrification inhibition was reflected in gradual releasing of ammonium nitrogen, which resulted in higher concentrations of ammonium nitrogen compared to concentrations of nitrate nitrogen at the beginning of maize growing season. The effect of both nitrification inhibitors in fertilizer ENSIN showed reduction processes and slowed down oxidation of ammonium nitrogen to nitrate nitrogen. The result was that considerably lower concentration of soil N-NO_3^- was observed in contrast to the soil N-NH_4^+ concentration in the plots treated with fertilizer ENSIN. The contents of nitrate nitrogen dominated over the contents of ammonium nitrogen at the end of maize growing season, when the nitrification inhibition activity ended. The highest contents of mineral nitrogen (N_{min}) were observed at fertilized treatment, where the nitrogen-sulphur fertilizer ENSIN with two nitrification inhibitors was applied.

The concentrations of N-NO_3^-, N-NH_4^+, as well as N_{min} in the soil were ranged at higher levels, as a result of higher application doses of nitrogen (160 kg*ha^{-1}). The highest contents of nitrate nitrogen in the soil were determined after the application of nitrogen fertilizer LAD 27 and nitrogen-sulphur fertilizer DASA 26/13 (both not containing inhibitors) during maize growing season, compared to contents of ammonium nitrogen. It is supposed that nitrification inhibitors slowed the disintegration of fertilizer in the soil and sequentially caused slower nitrogen releasing. It is necessary to include this particular effect in complex research from the perspective of field crops and all environmental aspects such as ground-water and N-NO_3^- leaching.

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