

## EFFECTIVENESS OF THE APPLICATION OF NITRIFICATION INHIBITORS ON THE CONTENT OF AVAILABLE NITROGEN FORMS IN THE SOIL AFTER WINTER BARLEY CULTIVATION

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The aim of this study was to examine the effect of split and one-time application of nitrogen-sulphur fertiliser ENSIN (26% N, 13% S) containing nitrification inhibitors (dicyandiamide and 1,2,4-triazole) on the content of nitrate and ammonium nitrogen in soil. The study was conducted in field small-plot experiment with winter barley on Haplic Luvisol with dominance of clay fraction in experimental station of the Central Testing Institute in Agriculture of Veľké Ripňany in the experimental years 2015 to 2017. The dose of nitrogen in all experimental treatments was 140 kg/ha and was applied at once during regenerative fertilisation or split in three partial doses. Soil samples from all examined treatments were taken from two soil depths (0.0–0.3 m and 0.3–0.6 m, respectively) by probe rod in three week intervals. Achieved results indicate that in the year 2015/2016, one-time application of fertiliser ENSIN containing nitrification inhibitors considerably reduced portion of  $\text{N-NO}_3^-$  from the content of  $\text{N}_{\text{min}}$  by 32 relative % in soil depth of 0.0–0.3 m and by 36 relative % in soil depth of 0.3–0.6 m. On the other hand, in the year 2016/2017, split application of fertiliser ENSIN substantially reduced portion of  $\text{N-NO}_3^-$  from the content of  $\text{N}_{\text{min}}$  by 38–62 relative % in soil depth of 0.0–0.3 m, however increased portion of  $\text{N-NO}_3^-$  by 10–14 relative % in soil depth of 0.3–0.6 m.

Key words: ammonium nitrogen, fertilisation, nitrates, nitrification, nitrification inhibitors

Nitrogen plays an important role on the plant growth and in the formation of yield of the agricultural crops (Fernandez-Escobar *et al.* 2011). Nitrogen as plant nutrient is usually used in greater quantities than those needed in order to guarantee a high yield in present practices (Sanchez *et al.* 1995). As a consequence, excessive application of nitrogen through fertiliser may cause environmental degradation due to nitrogen losses (Giménez *et al.* 2001). Nitrogen losses are caused by nitrate leaching (nitrification), erosion, volatilization, denitrification and fixation in soil organic matter (Marschner 1995). Nitrate leaching from agricultural soils and the contamination of water resources is one of the serious global environmen-

tal concerns (Di & Cameron 2002; Di & Cameron 2005; Liu *et al.* 2005; Elbl *et al.* 2014; Plošek *et al.* 2017). The high concentration of nitrate nitrogen in groundwater and drinking water can be a health hazard for humans (Knobeloch *et al.* 2000; Chen *et al.* 2003) and for livestock (Li *et al.* 2008).

The aim of developing management strategies and tools for reducing greenhouse gas emissions ( $\text{NO}_x$ ,  $\text{N}_2\text{O}$ ) and minimizing nitrogen loss from soils is very important. Adopting mitigation options at large scale by farmers requires simple and efficient tools and methods, as has been the case for nitrification inhibitors (Morales *et al.* 2015) or urease inhibitors (Musilová *et al.* 2012).

Nitrification inhibitors are a group of chemical

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compounds that suppress the first step of nitrification by inhibiting *Nitrosomonas* spp. bacteria that oxidize ammonium nitrogen to nitrite nitrogen, and therefore delay the nitrification process (Huber *et al.* 1977; Zerulla *et al.* 2001). Field studies have shown that the efficiency of nitrification inhibitors varies depending greatly on the environmental conditions (Menéndez *et al.* 2012). Inhibiting process of nitrification through use of nitrification inhibitor can encourage the retention of soil nitrogen as the less mobile ammonium form of nitrogen, which could significantly reduce leaching loss of nitrate nitrogen from the soil. Lower concentration of the soil nitrate nitrogen resulting from the inhibition of nitrification also reduces substrate availability for denitrification and decreases  $N_2O$  emissions (Akiyama *et al.* 2010).

The exploitation of activity of nitrification inhibitors could become an effective strategy contributing to the development of agronomic systems, benefiting both agriculture and the environment (Zhang *et al.* 2015).

Including the use for inhibiting nitrification in the soil, the nitrification inhibitor dicyandiamide is used as a basic chemical ingredient for the manufacture of industrial products, in electrical engineering and electronics, for the manufacture of ingredients in the form of food ingredients approved by the USFDA (OECD 2003).

In this study, we hypothesize that the application of nitrification inhibitors dicyandiamide and 1,2,4-triazole in the fertiliser ENSIN will delay microbial oxidation of ammonium nitrogen to nitrate nitrogen in the soil, as a consequence, the concentration of nitrate nitrogen in the soil will be lower.

## MATERIAL AND METHODS

Two-year small-plot experiment with winter barley growing was established on Haplic Luvisol with dominance of clay fraction in experimental station of the Central Testing Institute in Agriculture of Veľké Ripňany in the years 2015 to 2017. 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 sowing of winter barley 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 experimental year 2015/2016 sowing of winter barley was carried out at the end of October and the harvest was performed at the end of June. In experimental year 2016/2017 sowing of winter barley was carried out in the first decade of October and the harvest was performed at the end of June.

The scheme of winter barley fertilisation treatments and the doses of nitrogen applied per hectare at respective growth stages are stated in Table 5. The following fertilisers were applied in the field experiment: nitrogen-sulphur fertiliser Ammonium nitrate + Ammonium sulphate DASA 26/13 (26% N, 13% S) and fertiliser ENSIN (with the same content of nutrients as fertiliser 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 fertiliser ENSIN as its integral part. The advantages of fertiliser ENSIN usage in comparison with fertiliser DASA 26/13 are that ENSIN is applied in one dosage and re-application of the fertiliser is not needed.

The total nitrogen dose of 140 kg/ha via nitrogen-sulphur fertilisers DASA 26/13 and ENSIN was applied three times by split application during regenerative fertilisation, production fertilisation and quality fertilisation of winter barley, respectively. Total nitrogen dose of 140 kg/ha through fertilisers DASA 26/13 and ENSIN was also applied by only one-time application during regenerative fertilisation of winter barley (Table 5).

Each treatment was replicated four times and each plot was represented by the area of 10 m<sup>2</sup> (8.9 m × 1.125 m). The winter barley 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 and 0.3 to 0.6 m in the experimental years 2015 to 2017. There were four samplings conducted in an interval of three weeks during winter barley growing season in the experimental years 2015 to 2017 (Table 6).

The contents of ammonium ( $N-NH_4^+$ ) and nitrate ( $N-NO_3^-$ ) nitrogen (in 1% leachate of  $K_2SO_4$ ) were determined by the colour method with Nessler test solution and by the colour method with phenol

2,4-disulphonic acid (Kováčik 1997) in taken soil samples before sowing winter barley and during the vegetation of winter barley, respectively. The content of mineral nitrogen ( $N_{\min}$ ) in soil was calculated as the sum of ammonium ( $N-NH_4^+$ ) and nitrate ( $N-NO_3^-$ ) nitrogen content.

Nitrate and ammonium nitrogen contents in soil were statistically evaluated by analysis of variance (ANOVA), linear regression analysis using Statgraphics Plus PC software, version 5.1 and the differences between treatments were assessed by *LSD* test.

T a b l e 1

Review of average temperatures in locality of Veľké Ripňany

Month	2015/2016 [°C]	2016/2017 [°C]	Long term average [°C] 1981–2010	Difference 2015/2016 [°C]	Difference 2016/2017 [°C]
August	23.7EW	19.7N	19.6	+4.1	+0.1
September	16.5N	17.8W	15.8	+0.7	+2.0
October	10.0N	9.1N	9.9	+0.1	-0.8
November	6.0W	4.8N	4.9	+1.1	-0.1
December	2.6W	-0.4N	0.5	+2.1	-0.9
January	-0.9N	-7.2VC	-2.2	+1.3	-5.0
February	5.4EW	2.4W	-0.3	+5.7	+2.7
March	6.2N	8.1VW	4.2	+2.0	+3.9
April	11.1N	9.5N	10.1	+1.0	-0.6
May	16.1N	16.4N	15.2	+0.9	+1.2
June	20.6VW	21.4VW	18.4	+2.2	+3.0
October–June	8.6VW	7.1N	6.7	+1.9	+0.4

where: VC – very cold; N – normal; W – warm; VW – very warm; EW – extraordinary warm (Kožnárová & Klabzuba 2002)

T a b l e 2

Review of precipitation in locality of Veľké Ripňany

Month	2015/2016 [mm]	2016/2017 [mm]	Long term average [mm] 1981–2010	% of long term average	
				2015/2016	2016/2017
August	97.1W	45.2N	62.0	156.60	72.90
September	29.2N	42.9N	43.0	67.90	99.80
October	74.8W	64.5W	37.0	202.20	174.30
November	38.9N	47.2N	50.0	77.80	94.40
December	15.8VD	12.1VD	47.0	33.60	25.70
January	46.5W	19.2D	35.0	132.90	54.90
February	96.8EW	22.1N	34.0	284.70	65.00
March	13.5D	23.1N	31.0	43.50	74.50
April	28.9N	46.2N	41.0	70.50	112.70
May	76.7W	36.3N	55.0	139.50	66.00
June	18.0VD	19.5VD	70.0	25.70	27.90
October–June	409.9N	290.2VD	400.0	102.50	72.60

where: VD – very dry; D – dry; N – normal; W – wet; EW – extraordinary wet (Kožnárová & Klabzuba 2002)

The portion of nitrate and ammonium nitrogen from mineral nitrogen in the soil was evaluated as the indicator of efficiency of the inhibitory effect of the fertiliser ENSIN. Established portions of N-NH<sub>4</sub><sup>+</sup> or N-NO<sub>3</sub><sup>-</sup> were compared to achieved values after application of the fertilisers without nitrification inhibitors.

$$P = A/B \times 100$$

where: *P* – portion of nitrate or ammonium nitrogen from content of mineral nitrogen in soil [%],  
*A* – content of nitrate or ammonium nitrogen in soil [mg/kg],  
*B* – content of mineral nitrogen in soil [mg/kg].

The contents of available phosphorus and potassium (in leachate of Mehlich III) were determined by colorimetry and by the method of flame photometry, respectively (Mehlich 1984). The contents of available calcium and magnesium (in leachate of Mehlich III) were measured using the atomic absorption spectrophotometer (Mehlich 1984). The content of available sulphur was determined in the solution of ammonium acetate by colorimetry. The exchange soil acidity (pH<sub>KCl</sub>) was set in the leach-

ate of 0.2 M potassium chloride (KCl) (Fiala *et al.* 1999). The content of oxidable carbon – C<sub>ox</sub> was set by the method of Ľurina in taken soil samples before sowing winter barley (Ľurin 1966).

## RESULTS AND DISCUSSION

Average air temperature was higher than a long term normal by 0.4°C to 1.9°C during the winter barley growing season (October – June) in all two experimental years (Table 1). However, substantial differences were found in both amount and distribution of atmospheric precipitation influencing the final concentrations of ammonium (N-NH<sub>4</sub><sup>+</sup>) and nitrate (N-NO<sub>3</sub><sup>-</sup>) nitrogen in the agricultural soil. In experimental year 2016/2017, deficit of precipitation amounting was pronounced up to –109.8 mm during the winter barley growing season in comparison with long term normal (Table 2). The efficiency of nitrification inhibitor to retard nitrate leaching depends on soil properties, such as texture, organic matter, temperature (Zerulla *et al.* 2001; Ignacio *et al.* 2003) and moisture, pH, texture, organic carbon and mineral nitrogen (Barth *et al.* 2001; Shepherd

T a b l e 3

Agrochemical characteristic of soil in locality of Veľké Ripňany (0.0–0.3 m)

Year	[mg/kg]						C <sub>ox</sub> [%]	pH <sub>KCl</sub>
	N <sub>min</sub>	P	K	Ca	Mg	S		
2015/2016	7.6L	65.0M	201.0M	2,900M	257.0G	2.50VL	1.06L	6.54SA
2016/2017	14.8M	73.0G	208.0M	1,900L	348.0H	1.25VL	1.03L	6.84N

where: VL – very low content; L – low content; M – medium content; G – good content; H – high content; VH – very high content; pH<sub>KCl</sub>: SA – slightly acidic; N – neutral

T a b l e 4

Agrochemical characteristic of soil in locality of Veľké Ripňany (0.3–0.6 m)

Year	[mg/kg]						C <sub>ox</sub> [%]	pH <sub>KCl</sub>
	N <sub>min</sub>	P	K	Ca	Mg	S		
2015/2016	6.0L	24.0L	190.0M	3,700G	350.0H	2.5VL	0.57VL	6.51SA
2016/2017	14.7M	30.0L	163.0M	2,050M	404.0H	1.25VL	0.81L	6.76N

where: VL – very low content; L – low content; M – medium content; G – good content; H – high content; VH – very high content; pH<sub>KCl</sub>: SA – slightly acidic; N – neutral

*et al.* 2012). Irigoyen *et al.* (2003) reported that soil temperature is the most determining factor for the stability of nitrification inhibitors in the soils. The extent to which nitrification inhibitors inhibit N<sub>2</sub>O emissions and nitrate leaching is also dependent on factors such as the application rate, time and method of nitrification inhibitors application (Barth *et al.* 2008; Verma *et al.* 2008; O'Connor *et al.* 2012; Zaman & Nguyen 2012), field management practices (irrigation type, geometry and application method of ammonium-based fertilisers) (Shepherd *et al.* 2012).

Statistically significant differences of contents of ammonium and nitrate nitrogen in soil were found between treatments without application of nitrogen fertilisers (control) and treatment fertilised, where nitrogen was applied via nitrogen-sulphur fertilisers (Table 7). Highly significant difference of content of nitrate nitrogen was determined at treatment fertilised with fertiliser DASA 26/13, where the total nitrogen dose was applied three times by split ap-

plication, in comparison with fertiliser containing nitrification inhibitors ENSIN split application. Beltran-Rendon *et al.* (2011) reported nitrification inhibitors are compounds that effectively inhibit nitrification process when applied to soils in conjunction with ammonium fertilisers, such as urea or ammonium sulphate. Statistically non-significant differences of N-NO<sub>3</sub><sup>-</sup> contents were found between split application of ENSIN and one-time application of fertilisers DASA 26/13 and ENSIN. Increase of nitrate nitrogen contents after split application of DASA 26/13 was not statistically significant, compared to treatments, where the total nitrogen dose of 140 kg/ha was applied by one-time application via fertilisers DASA 26/13 and ENSIN. Statistically non-significant differences of ammonium nitrogen contents were found between DASA 26/13 and ENSIN, which were applied by split application and by one-time application during the winter barley growing season. The contents of ammonium and nitrate nitrogen were significantly affected by the ex-

T a b l e 5

Scheme of winter barley fertilisation treatments

Treatment	Fertiliser	Regenerative fertilisation BBCH 25 April 4, 2016 and March 17, 2017		Production fertilisation BBCH 32 May 2, 2016 and March 29, 2017		Quality fertilisation BBCH 49-51 May 19, 2016 and April 26, 2017	
		Doses of nutrients [kg/ha]					
		N	S	N	S	N	S
1	Control without fertilisation	–	–	–	–	–	–
2	DASA 26/13 split application	60	30	50	25	30	15
3	ENSIN split application	60	30	50	25	30	15
4	DASA 26/13 one-time application	140	70	–	–	–	–
5	ENSIN one-time application	140	70	–	–	–	–

T a b l e 6

Soil sampling dates during winter barley growing season

Soil sampling	Soil sampling dates	
	2015/2016	2016/2017
1 <sup>st</sup> sampling	May 2, 2016 (BBCH 32)	March 29, 2017 (BBCH 32)
2 <sup>nd</sup> sampling	May 24, 2016 (BBCH 51)	April 20, 2017 (BBCH 51)
3 <sup>rd</sup> sampling	June 10, 2016 (BBCH 83)	May 12, 2017 (BBCH 75)
4 <sup>th</sup> sampling	June 29, 2016 (BBCH 89)	June 2, 2017 (BBCH 87)

perimental years. The content of available nitrogen forms (N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>3</sub><sup>-</sup>) in the soil were significantly influenced by offtake of soil samples in soil depth of 0.0–0.3 m in both experimental years.

The lowest contents of nitrate (N-NO<sub>3</sub><sup>-</sup>), ammonium (N-NH<sub>4</sub><sup>+</sup>) and mineral nitrogen (N<sub>min</sub>) were observed at control treatment without application of nitrogen fertilisers in both experimental years. Relatively higher nitrate nitrogen contents in the soil were found after split application of DASA 26/13 and also after one-time application of DASA 26/13. On the contrary, the lowest values of nitrate nitrogen in soil were determined after application of ENSIN containing nitrification inhibitors, which was applied by split application and one-time application during the winter barley growing season. Similarly, Lam *et al.* (2015) state that, the nitrification inhibitor lowers nitrate leaching by preventing or slowing the microbial conversion of ammonium nitrogen to nitrate nitrogen in the soil.

The soil ammonium nitrogen contents ranged from 11.18 to 24.06 mg/kg in soil depth of 0.0–0.3 m and from 8.43 to 16.03 mg/kg in soil depth of 0.3–0.6 m (Table 8). The soil nitrate nitrogen contents ranged from 4.24 to 7.98 mg/kg in soil depth of 0.0–0.3 m and from 4.01 to 5.81 mg/kg in soil

depth of 0.3–0.6 m. One of the possible technologies to mitigating nitrogen losses such as nitrate leaching and N<sub>2</sub>O emissions is the application of nitrification inhibitors. Many nitrification inhibitors, such as dicyandiamide have been proven to be effective in reducing nitrification rates in soil (Gong *et al.* 2013). The presence of nitrification inhibitors in nitrogen-sulphur fertiliser ENSIN slowed down rate of nitrification process. Sivasakthy and Gnanaivelrajah (2012) state that, the use of the nitrification inhibitors may retain nitrogen in the ammonium form that is less susceptible to leaching and thus minimizes nitrogen loss. Thus, the addition of inhibitors nitrification dicyandiamide and 1,2,4-triazole maintained soil nitrogen in ammonium form of nitrogen longer time. The lowest values of nitrate nitrogen were found after one-time application of ENSIN in both soil depths. The effect of dicyandiamide and 1,2,4-triazole was reflected at treatment fertilised with fertiliser ENSIN, and consequently the ammonium portion from the content of N<sub>min</sub> was increased by 7 relative % in soil depth of 0.0–0.3 m and by 10 relative % in soil depth of 0.3–0.6 m after one-time application of ENSIN, in comparison with treatments, where DASA 26/13 was applied by three times split application and also one-time appli-

T a b l e 7

Effect of addition of nitrification inhibitors on the content of available nitrogen forms in the soil in 2015–2017

Treatment	Content of N [mg/kg]	
	N-NH <sub>4</sub> <sup>+</sup>	N-NO <sub>3</sub> <sup>-</sup>
Control	5.87 <sup>a</sup>	4.13 <sup>a</sup>
DASA 26/13 split application	17.96 <sup>b</sup>	6.95 <sup>c</sup>
ENSIN split application	16.61 <sup>b</sup>	5.98 <sup>b</sup>
DASA 26/13 one-time application	15.01 <sup>b</sup>	6.75 <sup>bc</sup>
ENSIN one-time application	16.32 <sup>b</sup>	6.50 <sup>bc</sup>
Experimental years		
Soil depth of 0.0–0.3 m	18.21 <sup>b</sup>	6.52 <sup>b</sup>
Soil depth of 0.3–0.6 m	10.49 <sup>a</sup>	5.60 <sup>a</sup>
<i>LSD</i> <sub>0.05</sub> treatment	4.36	0.79
<i>LSD</i> <sub>0.05</sub> year	2.76	0.50
<i>LSD</i> <sub>0.05</sub> soil depth	2.76	0.50

where: *LSD*<sub>0.05</sub> – least significant difference at the level  $\alpha = 0.05$  (*LSD* test); different letter behind a numerical value indicate statistically significant difference at the level 95.0%

cation. Simultaneously, the lowest nitrate nitrogen portion was determined after one-time application of ENSIN. The portion of  $\text{N-NO}_3^-$  was reduced by 32 relative % in soil depth of 0.0–0.3 m and by 36 relative % in soil depth of 0.3–0.6 m, compared to DASA 26/13, which was applied by split application and by one-time application. In other study, Panáková *et al.* (2016) determined that in the experimental year 2013, the soil nitrate nitrogen content under maize

growing was decreased by 9 relative % and the soil ammonium nitrogen content was increased by 18 relative % at treatment, where ENSIN was applied by one-time application, in comparison with treatments, where fertilisers LAD 27 and DASA 26/13 without inhibitors of nitrification were applied.

In experimental years 2016/2017, the contents of mineral nitrogen ranged from 7.10 to 30.40 mg/kg in soil depth of 0.0–0.3 m and from 6.30 to 21.10 mg/kg

T a b l e 8

Content of ammonium and nitrate nitrogen and portion of nitrogen forms from  $\text{N}_{\min}$  in the soil in 2015–2016 (average of sampling dates and replications)

Nutrition treatment	Soil depth [m]	Content of N [mg/kg]		$\text{N}_{\min}$ [mg/kg]	Portion of N from $\text{N}_{\min}$ in the soil [%]	
		$\text{N-NH}_4^+$	$\text{N-NO}_3^-$		$\text{N-NH}_4^+$	$\text{N-NO}_3^-$
Control	0.0–0.3	11.18	4.24	15.42	72	28
	0.3–0.6	8.43	4.01	12.44	68	32
DASA 26/13 split application	0.0–0.3	24.06	7.98	32.04	75	25
	0.3–0.6	13.43	5.81	19.24	70	30
ENSIN split application	0.0–0.3	23.30	6.54	29.84	78	22
	0.3–0.6	16.03	5.49	21.52	74	26
DASA 26/13 one-time application	0.0–0.3	19.35	6.50	25.85	75	25
	0.3–0.6	11.73	4.91	16.64	70	30
ENSIN one-time application	0.0–0.3	19.95	4.69	24.64	81	19
	0.3–0.6	15.43	4.29	19.72	78	22

T a b l e 9

Content of ammonium and nitrate nitrogen and portion of nitrogen forms from  $\text{N}_{\min}$  in the soil in 2016–2017 (average of sampling dates and replications)

Nutrition treatment	Soil depth [m]	Content of N [mg/kg]		$\text{N}_{\min}$ [mg/kg]	Portion of N from $\text{N}_{\min}$ in the soil [%]	
		$\text{N-NH}_4^+$	$\text{N-NO}_3^-$		$\text{N-NH}_4^+$	$\text{N-NO}_3^-$
Control	0.0–0.3	2.30	4.80	7.10	32	68
	0.3–0.6	2.00	4.30	6.30	32	68
DASA 26/13 split application	0.0–0.3	17.80	7.20	25.00	71	29
	0.3–0.6	10.30	5.90	16.20	64	36
ENSIN split application	0.0–0.3	23.90	6.50	30.40	79	21
	0.3–0.6	7.20	5.30	12.50	58	42
DASA 26/13 one-time application	0.0–0.3	15.30	8.00	23.30	66	34
	0.3–0.6	13.00	8.10	21.10	62	38
ENSIN one-time application	0.0–0.3	16.20	7.80	24.00	68	32
	0.3–0.6	9.10	8.30	17.40	52	48

in soil depth of 0.3–0.6 m (Table 9). The highest values of  $N-NH_4^+$ , as well as contents of  $N_{min}$  were determined at treatments fertilised with ENSIN, where the total nitrogen dose was applied by three times split application. Nitrification inhibitors dicyandiamide and 1,2,4-triazole which forms part of nitrogen-sulphur fertiliser ENSIN had positive effect on decrease of nitrate nitrogen concentration in the soil. The lowest contents of nitrate nitrogen were found after split application of ENSIN in both soil depths. Inhibitors of nitrification dicyandiamide and 1,2,4-triazole limited the nitrification process in the soil. The lowest nitrate nitrogen portion from the content of  $N_{min}$  was determined after three time split application of ENSIN. This resulted in a decrease of soil nitrate nitrogen concentration by 38 relative % in soil depth of 0.0–0.3 m, compared to DASA 26/13, which applied split and by 62 relative % in soil depth of 0.3–0.6 m, in comparison with treatment fertilised, where the total nitrogen of 140 kg/ha was applied by one-time application of fertiliser DASA 26/13. Split application of ENSIN increased portion of ammonium nitrogen from content of  $N_{min}$  by 10 relative % in soil depth of 0.0–0.3 m, in comparison with treatment fertilised with DASA 26/13 without nitrification inhibitors, where was applied by split application and by 16 relative % in soil depth of 0.0–0.3 m, compared to treatment fertilised with DASA 26/13, which was applied by one-time application during regenerative fertilisation. However, in soil depth of 0.3–0.6 m, split application of ENSIN decreased portion of  $N-NH_4^+$  by 10 relative %

and increased portion  $N-NO_3^-$  by 14 relative %, in comparison with split application of DASA 26/13. Also, split application of fertiliser ENSIN decreased portion of  $N-NH_4^+$  by 7 relative % and increased portion  $N-NO_3^-$  by 10 relative %, compared to one-time application of DASA 26/13. Panáková *et al.* (2016) published that on the average of four experimental years and three soil depths of the soil profile, one-time application of fertiliser ENSIN containing nitrification inhibitors substantially reduced portion of nitrate nitrogen from the content of mineral nitrogen in soil by 7–32 relative % during maize growing season. Liu *et al.* (2013) observed that the application of DCD and DMPP slightly decreased the soil  $N-NO_3^-$  contents and significantly increased the soil  $N-NH_4^+$  contents. The conversion processes between  $N-NH_4^+$  and  $N-NO_3^-$  (nitrification and denitrification) were inhibited by the nitrification inhibitors. Slamka and Ložek (2015) determined that, the presence of nitrification inhibitors in fertiliser ENSIN had been positive effect on slowing down of conversion ammonium nitrogen to nitrate nitrogen during oilseed rape growing season. One-time application of ENSIN substantially reduced  $N-NO_3^-$  portion from content of mineral nitrogen by 14 relative % in soil depth of 0.0–0.3 m and by 20 relative % in soil depth of 0.3–0.6 m. Qiao *et al.* (2015) determined that, on average, nitrification inhibitor reduced nitrate leaching (–47%, –59% to –32%). The reduction in nitrate leaching was significant for different types of ecosystem, fertiliser and soil texture. For ammonium leaching, the overall effect of nitri-

T a b l e 10

Effect of applied fertilisers on the grain yield of winter barley

Nutrition treatment	Grain yield [t/ha]	
	2015/2016	2016/2017
Control	4.44 <sup>a</sup>	4.58 <sup>a</sup>
DASA 26/13 split application	7.25 <sup>cd</sup>	8.69 <sup>bcd</sup>
ENSIN split application	5.15 <sup>b</sup>	8.70 <sup>cd</sup>
DASA 26/13 one-time application	6.68 <sup>c</sup>	8.33 <sup>b</sup>
ENSIN one-time application	7.85 <sup>d</sup>	8.85 <sup>d</sup>
<i>LSD</i> <sub>0.05</sub>	0.71	0.37

where: *LSD*<sub>0.05</sub> – least significant difference at the level  $\alpha = 0.05$  (*LSD* test); different letter behind a numerical value indicate statistically significant difference at the level 95.0%

fication inhibitors was not significant. The reduction in soil nitrate concentration under nitrification inhibitor addition resulted in a significant reduction in nitrate leaching. Although soil ammonium concentration increased under nitrification inhibitor application, ammonium leaching was not changed, probable because ammonium nitrogen is not as mobile as nitrate nitrogen. Overall, nitrification inhibitor application reduced total nitrogen loss by 16.5%, suggesting that nitrification inhibitor application is an effective practice to reduce nitrogen lost to environment. Gong *et al.* (2013) found out that, without DCD addition, soil content of ammonium nitrogen in treatment N declined sharply and was significantly lower than that in treatments N+DCD 1 and N+DCD 2 at day 7 and until the end of the experiment. Comparison with treatment N+DCD 1, a double dose of DCD maintained significantly higher levels of soil ammonium nitrogen from day 14 to the end of the experiment. In other research, Sivasakthy and Gnanavelrajah (2012) also determined that nitrate leaching from nitrification inhibitors treated pots were significantly less than relevant untreated pots. Significant reduction in nitrate leaching loss was observed between soil treatments with and without nitrification inhibitors. However, it should be aware that many factors, such as climate, soil types, cropping time, seasonality fertilisation and fertilisation method, would affect the value of nitrogen loss factor and eventually alter the estimation for the amount of nitrogen loss (Qiao *et al.* 2015).

The grain yields of winter barley are stated in Table 10. It can be seen from the table that presence of nitrification inhibitors in fertiliser ENSIN showed positive effect on grain yield because in the both experimental years the highest yield (7.85 t/ha and 8.85 t/ha, respectively) was achieved just in the treatment where ENSIN was applied in one-time. On the other side, split application of fertiliser ENSIN was not successful in the first experimental year (5.15 t/ha), but very effective in the second year of experiment (8.70 t/ha).

## CONCLUSIONS

The presence of nitrification inhibitors in nitrogen-sulphur fertiliser ENSIN had positive effect on

reduction of nitrate nitrogen concentration in soil. The common effect of dicyandiamide and 1,2,4-triazole was confirmed at treatments fertilised with fertiliser ENSIN, which was applied by one-time application or by split application during the winter barley growing season. The inhibitors retained soil nitrogen in the ammonium form ( $\text{N-NH}_4^+$ ) longer time. The application of ENSIN decreased the portion of nitrate nitrogen from the content of mineral nitrogen in soil by 14–62 relative % in comparison with fertiliser DASA 26/13, which was applied by split application and one-time application. The one-time application of ENSIN was more effective in experimental year 2015/2016. Oppositely, in the experimental year 2016/2017, split application of ENSIN was more effective in reducing nitrate nitrogen portion from content of mineral nitrogen in soil. This different effect of nitrification inhibitors in ENSIN was caused by various weather conditions in respective experimental years.

The nitrification inhibitors contributed to gradual releasing of ammonium nitrogen what caused higher concentrations of ammonium nitrogen compared to nitrate nitrogen in soil. The reduction of soil  $\text{N-NO}_3^-$  concentration subsequently resulted in a considerably decrease of  $\text{N-NO}_3^-$  leaching. The portion of ammonium nitrogen from the content of  $\text{N}_{\text{min}}$  in soil was increased by 3–16 relative % in comparison with fertiliser DASA 26/13, which was applied by split application and one-time application.

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