Original Paper

Effect of nitrogen and nitrogen-sulphur fertilization and inhibitors of nitrification on the yield and quality of maize grain

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Article Details: Received: 2016-10-23 | Accepted: 2016-03-03 | Available online: 2016-05-31

dx.doi.org/10.15414/afz.2016.19.02.45–50

Effect of split application of nitrogen and nitrogen + sulphur nutrition in comparison with one shot application of nitrogen + sulphur + inhibitors of nitrification (dikyandiamid and 1, 2, 4 triazol) on the yield and quality of maize grain was examined in three-year small-plot experiment on medium heavy degraded brownsoil. On the average of three experimental years one-shot application of nitrogen-sulphur nutrition in the form of ENSIN fertilizer containing nitrification inhibitors applied before maize seeding at the rate of 160 kg ha^{-1} N and 80 kg ha^{-1} S showed the most considerable effect on all investigated parameters. Yield of grain was increased highly significantly by 1.56 t ha^{-1} (+21.8%) compared to control treatment. Both parameters such as: thousand kernel weight (TKW) and content of crude protein were elevated by 7.9% and 8.3%, respectively. Production of protein per hectare was increased by 253 kg, that is by 31.9% and natural effectiveness of fertilization achieved the highest value, namely 9.8 kg of grain per 1 kg of applied nitrogen. When fertilizer ENSIN (contains inhibitors of nitrification) was applied in maize nutrition the achieved results were insignificantly better in all examined parameters in comparison with the split application of DASA 26/13 fertilizer under the same rate of both nitrogen and sulphur. There was found out positive tendency of favourable effect on yield and quality of grain of maize and decrease of application costs as a consequence of one shot application in this treatment. Addition of sulphur to nitrogen as well as an addition of inhibitors + sulphur to nitrogen significantly increased only grain yield of maize. Content of crude protein and TKW were increased insignificantly. Natural effectiveness of nitrogen fertilization was increasing in accordance with raising yields of maize grain as follows: nitrogen < nitrogen + sulphur < nitrogen + sulphur + inhibitors.

Keywords: growth stage, inhibitors, nitrification, nitrogen, split

1 Introduction

Globally increasing fertilization with nitrogen fertilizers contributed decisively to the rise of agricultural production (Dobermann and Cassmann, 2002; 2004). Losses of N via leaching and gaseous emissions generally increase with farming intensity (Ledgard, 2001) and so unless effective controls can be found to minimise these losses, they could put a limit on the productivity. In reality agricultural crops take up only around 50 % of antropogenic input N (Cassmann et al., 2002; Galloway et al., 2003) resulting in negative impact on ecological systems (nitrate leaching, eutrophisation, acidification, gaseous N emissions) and particularly adverse effect on the climate and the loss of biological soil diversity (Xi et al., 2010). For this reason a lot of research works deal with the reduction of these losses and more effective utilization of nitrogen fertilizers. Various possibilities for more effective utilization of fertilizer N (Ladha et al., 2005) are represented mainly by local specific fertilization strategies, more effective application methods and application of improved N-fertilizers fit out with inhibitors. The combination of ammonium or urea fertilizers with inhibitors which hinder activity of soil Nitrosomonas bacteria seems to be a successful way (Trenkel, 1997). The hydrolysis of urine and urea to ammonia is usually rapid (several days) and is facilitated by a ubiquitous soil microbial enzyme, urease. Major efforts have been made around the world to try to mitigate both NO_3^- leaching and N_2O emissions from agricultural land to meet national water quality standards or to fulfil international obligations of cutting greenhouse gas emissions under the Kyoto Protocol (Di and Cameron, 2002; Kramer et al., 2007). According to Malý et al. (2002) the amounts of ammonium ions in all the soils monitored were remarkably lower compared to the nitrate levels. The majority of the research indicates that nitrification inhibitors, when applied to soils in conjunction with N fertilizers or animal wastes, have beneficial effects on reducing nitrate leaching and nitrous oxide emissions, and, as a result, increase plant growth (Merino et al., 2002). The nitrification inhibitor dikyandiamid (DCD) decreases NO_3^- leaching by inhibiting the growth and activity of the ammonia-oxidizing bacteria in the soil, thus slowing down the rate of nitrification and keeping the N in the NH_4^+ form which is adsorbed onto the soil

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exchange surfaces and is available for plant uptake (Asing et al., 2008; Di et al., 2010). But, this is not always the case. There are reports of nil or variable effects of nitrification inhibitors on N losses and plant yields (Merino et al., 2002). Furthermore, there are some reports suggesting that some nitrification inhibitors may have a toxicity effect on some plants (Macadam et al., 2003). However, DCD is considered one of the most environmentally-benign nitrification inhibitors. Thus, both urease inhibitors and nitrification inhibitors can be tools to manage N loss profitably (Laboski, 2006).

Maize has quite great demands for nitrogen nutrition, but lower requirements for sulphur fertilization at the same time. One ton of maize grain and respective amount of maize straw take away 22 to 30 kg N and 2.7 to 3.5 kg S from soil depending on hybrid and soil-climatic condition of stand (Vaněk et al., 2007; Zimolka et al., 2008; Fecenko and Ložek, 2000). Under the deficit of nitrogen in soil, its content in plants of maize decreases considerably. The plants develop poorly and the leaves are light-green coloured. According to the degree of the deficiency the colour of leaves changes from light-green up to yellow with typical shape of letter "V" oriented towards basal part of leaf (Richter and Ryant in Zimolka et al., 2008). In consequence of this both the number of grains in maize cob and thousand kernel weight decrease resulting in grain yield reduction and grain quality worsening.

As corn growers may reduce N rates because of high N prices, urease and nitrification inhibitors may play a larger role in providing insurance against yield reductions should N losses occur (Laboski, 2006).

Both urease inhibitors and nitrification inhibitors can be tools to manage N loss profitably in today's economic climate. In order to insure the greatest probability of positive economic returns with these materials, it is important to know what environmental and management conditions increase the risk of N loss (Laboski, 2006).

While nitrification inhibitors alone reduced N_2O emissions at similar levels compared to the combined urease and nitrification inhibitors; unlike nitrification inhibitor alone, the combined urease plus nitrification inhibitors can also reduce urea-induced NH_3 and N_2O losses following either method of application. However, further investigation under field conditions is necessary to determine whether coupled inhibitors are environmentally benign and suitable to achieve optimum yields by adopting crop-specific appropriate method and timing of fertilization (Khalil et al., 2009).

Under this context, the aim of this study was to determine effects of nitrogen, nitrogen-sulphur nutrition and inhibitors of nitrification on the yield and quality of maize grain and natural effectiveness of nitrogen fertilization at both split and one-shot fertilizer application.

2 Material and methods

Three-year small-plot experiment with grain maize (hybrid, Chapalu FAO 350) 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 years 2012 to 2014. In respective years, experimental site was rotated within farm crop rotation. Current temperatures and precipitations in respective experimental years as well as long term normals are stated in Table 1 and 2.

Soil samles for agrochemical analyses were taken before seeding of maize from soil depth of 0.0 to 0.30 m. Agrochemical characteristics of soil samples and their evaluations are stated in Table 3. Scheme of maize fertilization treatments and the doses of nitrogen applied per hectare at respective growth stages are stated in Table 4. There were applied the following fertilizers in the experiment: LAD 27 (27% N), DASA 26/13 (26% N, 13% S), ENSIN (26% N, 13% S and two inhibitors of nitrification). Inhibitors of interest were represented by dikyandiamid (DCD) and 1, 2, 4 triazol (TZ) which were incorporated directly in the granule of fertilizer ENSIN as its integral part.

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

Month	2012	2013	2014	30-year normal	Difference 2012	Difference 2013	Difference 2014
January	1.4	-1.1	2.7	-2.1	+3.5	+1	+4.8
February	-3.0	1.5	4.6	0.0	-3.0	+1.5	+4.6
March	7.5	3.5	8.9	5.0	+2.5	-1.5	+3.9
April	12.9	13.0	13.0	9.6	+3.3	+3.4	+3.4
May	17.6	17.2	16.4	15.1	+2.5	+2.1	+1.3
June	21.3	20.7	21.5	17.9	+3.4	+2.8	+3.6
July	23.1	24.6	23.5	19.9	+3.2	+4.7	+3.6
August	22.2	23.7	19.8	19.3	+2.9	+4.4	+0.5
September	17.6	14.8	17.2	14.7	+2.9	+0.1	+2.5

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

Month	2012	2013	2014	30-year normal	Difference 2012	Difference 2013	Difference 2014
January	34	83	37	41	-7.0	+42	-4
February	37.5	66	56	30	+7.5	+33	+26
March	0	93	21	37	-37	+56	-16
April	46	24	37	39	+7.0	-15	-2
May	15	145	84	59	-44.0	+86	+25
June	31	116	35	68	-37.0	+48	-33
July	86	16	89	48	+38.0	-32	+41
August	0	55	124	47	-47.0	+8	+77
September	35	55	79	42	-7.0	+13	+37
October	80.5	-	22	40	+40.5	-	-18
Apr.–Sept.	213	411	448	303	-90.0	+108	+145

Each treatment was 4 times repeated and each plot was represented by the area of 100 m^2 (20 m × 5 m). Harvest of grain was performed at the beginning of November under the grain moisture of 16.5; 15.0 and 17.3% in respective experimental years. Grain yield was re-calculated to standard moisture of 14%. Samples of grain for protein content and thousand kernel weight determination were taken immediately after harvest.

Content of crude protein in grain was calculated as follows:

$$\textit{Content of protein}\ (\%) = \textit{content of nitrogen in grain}\ (\%) \times 6.25$$

Content of nitrogen in grain was determined by Kjeldahl method (Cohen, 1910).

Production of protein per hectare was calculated according to Equation:

$$\textit{Production of protein per hectare}\ (\text{t ha}^{-1}) = \textit{yield}\ (\text{t ha}^{-1}) \times \textit{content of crude protein in grain}\ (\%) / 100 \times 0.86$$

where:
0.86 = convertion to standard dry matter content at standard moisture content of maize grain (14%)

The effect of applied fertilizers on grain yields was evaluated from economical point of view by coefficient of natural effectivness (K_{NE}):

$$K_{NE} = \Delta U / DN$$

where:

ΔU – increment of grain yield per hectare due to fertilization in comparison to control unfertilized treatment

DN – dose of nitrogen per hectare in respective treatments

The grain yield of maize, content of protein and thousand kernel weight of grain were statistically evaluated by analysis of variance and the differences between treatments of fertilization were assessed by Tukey test.

3 Results and discussion

Temperature of air was higher by 2.5 to 3.0 °C than longterm normal during growing season of maize in all three experimental years (Table 1). However, considerable differences were found in both sum and distribution of atmospheric precipitation influencing final grain yield (Table 2). In 2014 the highest yield of grain (9.73 t ha^{-1}) was achieved on the average of four treatments when precipitation represented +145 mm surplus against longterm normal. On the contrary, deficit of precipitation amounting -90 mm in comparison to longterm normal caused yield depression by 17.3% in year 2012. In spite of surplass (+108 mm), strong irregularity of precipitation distribution during the whole growing season of maize caused decrease of grain yield even by 32.1% in year

Table 3 Content of macronutrients in soil (0.0–0.3 m)

Year	Mg (kg^{-1})	P	K	Ca	Mg	S	Humus (%)	pH / KCl
	N_{min}							
2012	29.9 D	42.5 N	301 V	2,300 S	301 V	12.5 N	2.73 S	5.78
2013	29.4 D	160 V	385 V	2,780 S	439 VV	27.5 S	3.54 D	6.92
2014	30.4 D	45 N	201 D	3,580 D	431 VV	1.9 VN	3.09 D	6.16

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

Table 4 Scheme of fertilization treatments

Treatment	Fertilizer	Before seeding		At seeding		Maize height of 0.3 m	
		Dose of nutrients (kg ha^{-1})					
		N	S	N	S	N	S
1	–	0	0	0	0	0	0
2	LAD 27	60	0	60	0	40	0
3	DASA 26/13	60	30	60	30	40	20
4	ENSIN	160	80	0	0	0	0

2013 in comparison to year 2014. On the average of three years split nitrogen fertilization in the form of LAD 27 fertilizer at total rate of 160 kg ha^{-1} (60 kg N + 60 kg N + 40 kg N – Table 4) highly significantly increased maize grain yield by 0.96 t ha^{-1}, that is by 13.4% in comparison to control treatment (Table 5).

At the same time, thousand kernel weight (TKW) was significantly increased by 17.6 g that is by 6.1% (Table 9) and also content of crude protein was significantly increased by 5.6% (Table 7). However, production of protein per hectare was increased even by 19.8% in comparison to unfertilized treatment (Table 8). Natural effectiveness of nitrogen fertilization represented 6.0 kg of maize grain per 1 kg of applied nitrogen (Table 6). Favourable effect of nitrogen nutrition on both the quantity and quality of maize grain is in accordance with the findings of many authors (Fecenko and Ložek, 2000; Vaňek et al., 2013; Hanáčková and Žembery, 2015).

Applied split N – S nutrition in the form of DASA 26/13 fertilizer at total rate of N 160 kg ha^{-1} and 80 kg ha^{-1} S (before seeding 60 kg N + 30 kg S ha^{-1}, at seeding 60 kg N + 30 kg S ha^{-1} and at the height of maize of 0.3 m 40 kg N + 20 kg S per hectare – Table 4) statistically highly significantly increased grain yield of maize by 1.39 t ha^{-1} that is by 19.4% in comparison to unfertilized control on the average of three experimental years (Table 5). In this case thousand kernel weight was also increased highly significantly by 20.7 g that is by 7.2% (Table 9) as well as content of crude protein which was increased by 7.0% (Table 7). Protein production per hectare was increased even by 28.2% (Table 8).

Natural effectiveness of this fertilization achieved the value of 8.7 kg of grain per 1 kg of applied nitrogen. From the above stated it is evident that addition of sulphur of 80 kg ha^{-1} to nitrogen nutrition (160 kg ha^{-1}) favourably influenced all examined parameters in comparison to solo nitrogen nutrition in treatment 2 as follows: grain yield was increased significantly by 0.43 t ha^{-1} (by 5.3%), thousand kernel weight was increased insignificantly by 3.1 g (by 1.0%), content of crude protein was also increased insignificantly by 1.3%. However, production of protein per hectare was incerased by 66 kg, that is

Table 5 Effect of applied fertilizers on grain yields of maize

Treatment	Yield of grain (t ha^{-1})				Relatively (%)		
	2012	2013	2014	3-year average	“1“ = 100 %	“2“ = 100 %	“3“ = 100 %
1 – unfertilized	6.78	6.02	8.64	7.15	100	–	–
2 – LAD 27	8.06	6.48	9.78	8.11	113.4^{++}	100	–
3 – DASA 26/13	8.64	6.84	10.14	8.54	119.4^{++}	105,3^{+}	100
4 – ENSIN	8.70	7.08	10.35	8.71	121.8^{++}	107.4^{++}	102.0^{-}
$LSD_{0.05}$	0.40^{+}	0.34^{+}	0.35^{+}	0.37^{+}	5.20^{+}	–	–
$LSD_{0.01}$	0.56^{++}	0.48^{++}	0.49^{++}	0.52^{++}	7.30^{++}	–	–

LSD – least significant difference

Table 6 Natural effectiveness values of maize fertilization (kg kg^{-1})

Treatment	Natural effectiveness of fertilization					
	2012	2013	2014	3-year average	“2“ = 100%	“3“ = 100%
1 – unfertilized	–	–	–	–	–	–
2 – LAD 27	8.0	2.9	7.1	6.0	100	–
3 – DASA26/13	11.6	5.1	9.4	8.7	145	100
4 – ENSIN	12.0	6.6	10.7	9.8	163	113

Table 7 Effect of applied fertilizers on protein content in maize grain

Treatment	Content of protein in grain (%)				Relatively (%)		
	2012	2013	2014	3-year average	"1" = 100 %	"2" = 100 %	"3" = 100 %
1 – unfertilized	15.29	11.97	11.58	12.95	100	–	–
2 – LAD 27	16.23	12.83	11.97	13.68	105.6+	100	–
3 – DASA 26/13	16.40	13.04	12.13	13.86	107.0+	101.3-	100
4 – ENSIN	16.52	13.26	12.28	14.02	108.3++	102.5-	101.2-
$LSD_{0.05}$	0.78+	0.62+	0.60+	0.65+	5.02+	–	–
$LSD_{0.01}$	1.09++	0.87++	0.84++	0.91++	7.03++	–	–

LSD – least significant difference

Table 8 Effect of aplied fertilizers on protein production per unit area

Treatment	Production of protein (t ha^{-1})				Relatively (%)		
	2012	2013	2014	3-year average	"1" = 100 %	"2" = 100 %	"3" = 100 %
1 – unfertilized	0.892	0.620	0.864	0.792	100	–	–
2 – LAD 27	1.125	0.715	1.007	0.949	119.8	100	–
3 – DASA26/13	1.219	0.767	1.058	1.015	128.2	107.0	100
4 – ENSIN	1.236	0.807	1.093	1.045	131.9	110.1	103.0

Table 9 Effect of fertilization on thousand kernel weight (TKW) of maize

Treatment	TKW (g)				Relatively (%)		
	2012	2013	2014	3-year average	"1" = 100 %	"2" = 100 %	"3" = 100 %
1 – unfertilized	281.1	295.5	288.5	288.4	100	–	–
2 – LAD 27	313.7	306.6	297.7	306.0	106.1+	100	–
3 – DASA 26/13	319.6	308.4	299.3	309.1	107.2++	101.0-	100
4 – ENSIN	325.0	308.6	299.8	311.1	107.9++	101.7-	100.6-
$LSD_{0.05}$	15.0+	14.8+	14.2+	14.6+	5.06+	–	–
$LSD_{0.01}$	21.0++	20.7++	19.9++	20.4++	7.08++	–	–

LSD – least significant difference

by 7.0% and natural effectiveness of fertilization was elevated by 2.7 kg of maize grain. Positive effect of sulphur fertilization on the yield and quality parameters of cereals grain was also found out by many other authors (Bergmann and Neubert, 1976; Ivanič et al, 1984; Richter and Hlušek, 1994).

On the average of three experimental years one-shot application of nitrogen-sulphur nutrition in the form of ENSIN fertilizer containing nitrification inhibitors DCD and TZ applied before maize seeding in treatment 4 at the rate of 160 k ha^{-1} N and 80 kg ha^{-1} S showed the most considerable effect on all parameters investigated in this experiment. Yield of grain was increased highly significantly by 1.56 t ha^{-1} (+21.8%) against control treatment. Both parameters TKW and content of crude protein were elevated by 7.9% and 8.3%, respectively. Production of protein per hectare was increased by 253 kg, that is by 31.9% and natural effectiveness of fertilization achieved the highest value, namely 9.8 kg of grain per 1 kg of applied nitrogen.

When fertilizer ENSIN (contains inhibitors of nitrification) was applied in maize nutrition the achieved results were insignificantly better in all examined parameters in comparison with the split application of DASA 26/13 fertilizer under the same rate of both nitrogen and sulphur. There was found out positive tendency of favourable effect on yield and quality of maize grain and decrease of application costs as a consequence of one shot application in this treatment. The problematic related to application of nitrification inhibitors into the soil was investigated by several authors (Marendiak et al., 1987; Slamka et al., 2014; Di and Cameron, 2004) who found out analogical effects of these active substances in their experiments.

4 Conclusions

Applied differentiated nutrition significantly increased grain yield of maize in the following graduating order: nitrogen < nitrogen + sulphur < nitrogen + sulphur + nitrification inhibitors (DCD + TZ). Nitrogen applied solo significantly increased content of crude protein in maize grain and thousand kernel weight as well. Combination sulphur + nitrogen as well as sulphur + nitrogen + nitrification inhibitors increased these parameters even highly significantly. Addition of sulphur to nitrogen as well as an addition of inhibitors + sulphur to nitrogen significantly increased only grain yield of maize (compared to treatment 2: LAD 27). Content of crude protein and thousand kernel weight were increased insignificantly. Natural effectiveness of nitrogen fertilization was increasing in accordance with raising yields of maize grain as follows: nitrogen < nitrogen + sulphur < nitrogen + sulphur + inhibitors (DCD + TZ).

Acknowledgments

This work was co-funded by European Community under project no 26220220180 Building Research Centre AgroBioTech and by project VEGA 1/0513/12 Research of agroecosystems for mitigation of climatic changes, production of bioproducts and an improvement of nutritional and healthy parameters of people.

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