EFFECT OF UREA CONTAINING INHIBITORS ON NITRATE PORTION IN SOIL AND GRAIN YIELD OF WINTER WHEAT *(TRITICUM AESTIVUM* L.)

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Abstract: *The objective of this study was to determine the effectiveness of urea containing inhibitor of nitrification (Dicyandiamide, DCD) or inhibitor of urease (1, 2, 4 Triazole) or both on nitrate nitrogen ratio of mineral nitrogen content in soil in 4-year small-plot experiment with winter wheat (variety ´Venistar´) on heavy brownsoil. Effect of inhibitors was evaluated as* $N\text{-}NO_3^-/\ N_{min}$ *ratio in respective soil profiles. Total dose of nitrogen (140 kg* ha^{-1}*) was applied in the form of urea at one shot (with inhibitors) or split to two (with inhibitors) or three partial rates (without inhibitors) in crucial growth stages of wheat. Soil samples were taken from the soil profiles of 0.0-0.30 m, 0.30-0.60 m and 0.60-0.90 m, respectively. Also yield of grain of wheat and natural effectiveness of fertilization were assessed. Achieved results indicate that application of inhibitors contained in urea significantly reduced* $N\text{-}NO_3^-/\ N_{min}$ *ratio in soil by 11.7% (urea+inhibitor of nitrification), 10.8% (urea+both inhibitors) and 9.4% (urea+inhibitor of urease) in comparison to urea applied without inhibitors. Adverse effect of applied inhibitors on the winter wheat grain yield was not found out. These results suggest that the both examined inhibitors contained in urea fertilizer have a potential to reduce portion of nitrates on mineral nitrogen content in soil. The highest coefficient of natural effectiveness of fertilization was achieved in treatment fertilized with urea containing both inhibitors (6.860), that is there was created 6.860 kg of wheat grain per each kilogramme of applied fertilizer nitrogen in this treatment. It was owing to the fact that urea with inhibitors was applied at one shot without splitting to partial doses during growing season in comparison to application of pure urea at which the same total dose of nitrogen was split to three partial doses. Both urease inhibitor and nitrification inhibitor can be tool to manage N loss profitably in today's economic climate.*

Key words: *ammonia, growth stage, nitrification, split application, urea, urease*

INTRODUCTION

Globally increasing fertilization with nitrogen fertilizers contributed decisively to the rise of agricultural production (DOBERMANN, 2005). 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 (BEEVER et al., 2007). 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 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 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.

MATERIALS AND METHODS

Four-year small-plot experiment with winter wheat (variety 'Venistar') was established on medium heavy degraded chernozem of locality Sládkovičovo - Nový Dvor (Breeding company HORDEUM, s.r.o. Sládkovičovo), Slovakia.

Agrochemical soil characteristics are stated in tables 1 and 2. Seeding rate represented five million germinative grains per hectare and seeding was realized at the beginning of October in each experimental year. Preventive spraying against aphids by pesticide VAZTAK 10 EC at the rate of 0.15 l per hectare was done in autumn to prevent potential transfer of viruses on plants. Chemical control against weeds was done using herbicide MUSTANG at the rate of 0.6 l ha^{-1} at the early beginning of April.

Table 1

Content of macronutrients in soil profile of 0.0-0.3 m before experiment with winter wheat establishment (locality of Sládkovičovo)

Year	N_{min}	P	K	Ca	Mg	S
	mg kg^{-1} of soil					
2009	9.2 L	85 H	365 VH	5500 H	370 VH	14 L
2010	9.5 L	82 H	365 VH	5800 H	355 VH	12.5 L
2011	9.2 L	85 H	340 VH	5600 H	350 VH	11 L
2012	14 M	85 H	270 G	7150 VH	399 VH	15 L

Note: L = low, M = medium, G = good, H = high, VH = very high

Pre-seeding fertilization of winter wheat in autumn was not realized for there was sufficient natural supply of N_{min} in soil needed for wheat growth during autumn period. Regeneration fertilization was realized at the start of winter wheat tillering at the beginning of April in treatments 2 to 6. Productional fertilization of treatments 2, 3 and 5 was realized at the start of growth stage of shooting in the third decade of April. Quality dressing was done only in treatment 2 by PIAGRAN 46 (normal urea without inhibitor of nitrification) and treatment 3 by LAD-27 around the 10th of May closely before flowering. Scheme of winter wheat fertilization treatments and concrete nitrogen rates applied per hectare at respective growth stages of winter wheat are stated in table 3.

Table 2

Content of micronutrients, humus and pH in soil profile of 0.0-0.3 m before experiment with winter wheat establishment (locality of Sládkovičovo)

Year	Zn	Fe	Mn	Cu	Humus	pH_{KCl}
	mg kg^{-1} of soil				%	
2009	1.25 M	8.55 M	4.20 L	2.15 G	2.85 M	7.45
2010	1.45 M	9.70 M	5.15 L	2.40 G	2.95 M	7.38
2011	1.60 M	15.0 M	7.50 L	2.50 G	2.80 M	7.57
2012	0.90 L	15.6 M	3.70 L	1.30 M	3.21 G	7.33

Note: L = low, M = medium, G = good, H = high, VH = very high

Table 3

Scheme of fertilization treatments with urea in winter wheat

Treatment	Fertilizer	Regeneration fertilization BBCH 32-33 (start of April)	Production fertilization BBCH 32-33 (3rd decade of April)	Quality fertilization BBCH 49-51 (2nd decade of May)
		kg ha^{-1} N		
1	without N	-	-	-
2	PIAGRAN 46 (U)	60	50	30
3	LAD 27 (LAD)	60	50	30
4	ALZON (U + IN)	140	-	-
5	PIAZUR (U + IU)	60	80	-
6	U + IN + IU	140	-	-

PIAGRAN 46 – normal urea
ALZON 46 – urea with inhibitor of nitrification
PIAZUR – urea with inhibitor of urease
U + IN + IU – urea with inhibitor of nitrification and urease
LAD 27 – ammonium nitrate + dolomite

Each treatment was 4 times repeated and was represented by the area of 10 m^2 (8 m x 1.25 m). Fertilizers in respective growth stages were applied by hand.

Soil samples from all examined treatments were taken by probe rod from the soil profiles of 0.0-0.30 m, 0.30-0.60 m and 0.60-0.90 m, respectively. On the course of winter wheat growing season 6 samplings of soil was done in 3-4 weeks intervals starting from the date of the first urea application (at the beginning of April at growth stage of the commencement of tillering of winter wheat) and ending at the full harvest maturity of winter wheat grain.

Content of ammonium nitrogen (colorimetrically by means of Nessler agent) and content of nitrate nitrogen (colorimetrically using phenol 2, 4- disulphonic acid) were determined in soil samples taken from respective soil profiles. Ratio of nitrate nitrogen content to content of mineral nitrogen in soil was chosen as a criterion of applied inhibitors effect and calculated as follows: r = A / B, where

r = ratio of nitrate nitrogen content to the content of mineral nitrogen in soil
A = content of nitrate nitrogen in soil (mg kg^{-1})
B = content of mineral nitrogen in soil (mg kg^{-1})

Content of mineral nitrogen in soil was calculated as the sum of ammonium and nitrate nitrogen content in soil (B = $N\text{-}NH_4^+$ + $N\text{-}NO_3^-$)

Harvest of winter wheat grain yield was realized by small-plot combine of Wintersteiger brand (Austria) around the 20th of July, in each respective year.

The grain yields were evaluated from economical point of view as well. Coefficient of natural effectiveness was calculated as follows: $K_{NE}=\Delta U/D_N$, where
ΔU = increment of grain yield per hectare due to fertilization in comparison to control unfertilized treatment
D_N = dose of nitrogen per hectare in respective treatments

Effect of examined inhibitors was investigated by application of urea which contained inhibitor of nitrification DCD (Dicyandiamide) and is refered here as ALZON or inhibitor of urease 1, 2, 4 Triazole (indicated as PIAZUR) or both these inhibitors (table 3).
Ratio of nitrate nitrogen content to the content of mineral nitrogen in soil and the grain yield of winter wheat were statistically evaluated by analyze of variance and the differences between treatments were assessed by Tukey test.

RESULTS AND DISCUSSION

Four-year averages of ammonium nitrogen ($N\text{-}NH_4^+$) and nitrate nitrogen ($N\text{-}NO_3^-$) contents in fertilized treatments in respective investigated depths of soil profile and in respective sampling dates are stated in table 4. On the basis of these data $N\text{-}NO_3^-/N_{min}$ ratio in respective soil depths was calculated (table 5). This table shows that on the average of four years and sampling dates the effect of applied inhibitors (treat. U+IN, U+IU, U+IN+IU) was manifested in the depths of 0.0-0.3 m significantly compared to treatment U and LAD in which inhibitors were not applied. In this depth the lowest $N\text{-}NO_3^-/N_{min}$ ratio (0.432) was found out in treatment fertilized by urea containing inhibitor of urease (treat. U+IU) and the difference against the value of $N\text{-}NO_3^-/N_{min}$ ratio in treatment U+IN (0.439) was not statistically significant, but in comparison to treatment U+IN+IU (0.484) the difference was calculated as significant. The highest content of nitrate nitrogen in relation to N_{min} content in soil was found in treatment fertilized with LAD-27 (0.721) which represents increase by 26.9% compared to application of „pure“ urea (treat. U) – table 6.

Thus, in treatments fertilized by urea with inhibitors decrease of $N\text{-}NO_3^-/N_{min}$ ratio was registered, namely by 22.7% (treat. U+IN), 23.9% (treat. U+IU) and 14.8% (treat. U+IN+IU) in comparison to treatment fertilized with urea without inhibitors (treat. U).

DI AND CAMERON (2012) stated that the application of DCD or DMPP (3, 4-Dimethylpyrazole phosphate) maintained lower NO_3^- concentrations in the soil between days 35 and 84 in both the 0–0.075 m depth and the 0.075–0.15 m depth compared with the urine alone treatment. The reductions in NO_3^- leaching losses by the nitrification inhibitors were equivalent to 36% with DCD and 28% with DMPP.

Value of origin $N\text{-}NO_3^-/N_{min}$ ratio before start of fertilization (0.669) was exceeded only in treatment fertilized with LAD-27, that is by fertilizer which contains nitrogen also in nitrate form, what refers to the fact that this fertilizer increases portion of nitrates in arable soil layer even in comparison with application of „pure“ urea without inhibitors. In addition, accordingly to MCGEOUGH et al. (2012) N_2O emissions were found to be predominantly associated with the NO_3^- pool, the effect of DCD in lowering N_2O emissions is limited in the presence of a NO_3^- fertilizer. To obtain the maximum cost-benefit of DCD in lowering N_2O

emissions, under mild moist conditions, its application should be restricted to ammonium based organic or synthetic fertilizers.

Table 5

Nitrate nitrogen content/mineral N content ratio in soil (average of sampling dates, years and repetitions)

Treatment	Depth of soil (m)		
	0.0 – 0.30	0.30 – 0.60	0.60 – 0.90
Before fertilization*	0.669	0.685	0.69.60
U	0.568 a	0.553 a	0.628 a
LAD 27	0.721 b	0.724 b	0.704 b
U + IN	0.439 cd	0.566 a	0.541 c
U + IU	0.432 c	0.558 a	0.595 d
U + IN + IU	0.484 d	0.544 a	0.530 c
$LSD_{0,05}$	0.050	0.054	0.054

Note: *original ratio of $N\text{-}NO_3^-/N_{min}$ in soil at the date of fertilization (these values were not included in the average calculation because they were the same for all treatments)

Table 6

Increase/decrease of $N\text{-}NO_3^-/N_{min}$ ratio in soil (%)

Depth (m)	U	LAD	U + IN	U + IU	U + IN + IU
0.00 - 0.30	100	+26.9	-22.7	-23.9	-14.8
0.30 - 0.60	100	+30.9	+2.3	+0.9	-1.6
0.60 – 0.90	100	+12.1	-13.9	-5.3	-15.6

In the depth of 0.30-0.60 m of soil profile the situation was different. Inhibition effect of inhibitors contained in urea was manifested only in treatment fertilized with urea containing both inhibitors (treat. U+IN+IU), but statistically insignificantly compared to pure urea application (table 5). The values of $N\text{-}NO_3^-/N_{min}$ ratio in all three treatments with application of urea containing inhibitors fluctuated around 0.550 what is practically identical with the value achieved in treatment fertilized by pure urea (0.553). One of the reasons of this phenomenon could be leaching of NO_3^- from upper soil horizon into lower soil profile of 0.30-0.60 m.

Effect of LAD-27 application on the increase of $N\text{-}NO_3^-/N_{min}$ ratio was in this soil horizon even higher than in previous upper depth. Ratio of $N\text{-}NO_3^-/N_{min}$ in this treatment increased even by 30.9% comparing to treatment U (table 6).

In opposition to soil profile of 0.0-0.30 and 0.60-0.90 m, respectively in the profile of 0.30-0.60 m no significant effect of inhibitors on $N\text{-}NO_3^-/N_{min}$ ratio was found in comparison with treatment U where urea without inhibitors was applied. There are reports of nil or variable effects of nitrification inhibitors on N losses and plant yields (Merino et al., 2002). The effectiveness of nitrification inhibitors may be affected by environmental and soil properties (Di and Cameron, 2004) which are also dependent on depth of analyzed soil profile and soil temperature (Irigoyen et al., 2003). Goos (2008) found out that nitrification rate was not slowed by adding Nutrisphere-N (fertilizer additive, which is claimed to prevent nitrogen loss from soil through the inhibition of urease and nitrification) to urea and only slightly reduced ammonia volatilization.

Also in this soil profile (0.30-0.60 m) the value of original $N\text{-}NO_3^-/N_{min}$ ratio before fertilization (0.685) was exceeded only in treatment LAD fertilized with fertilizer containing nitrates (0.724).

It results from table 5 that $N\text{-}NO_3^-/N_{min}$ ratio in soil profile of 0.30-0.60 m was higher on treatments with inhibitors in comparison with the same treatments in the depth of 0.00-0.30

m. It could be caused by NO_3^- shift into the lower soil horizons increasing their concentration relatively to mineral nitrogen content.

Effect of inhibitors contained in urea was manifested also in the depth of 0.60-0.90 m; and the manifestation was partly in similar way like in 0.00-0.30 m horizon. The highest inhibitor effect was determined in treatment fertilized with urea containing both inhibitors (U+IN+IU) in which the ratio of $N\text{-}NO_3^-/N_{min}$ achieved value 0.530 what represents statistically significant decrease in comparison with treatment U (0.628), treatment LAD (0.704) and also with treatment U+IU (0.595). Statistically insignificant difference was found only in comparison with treatment fertilized with urea containing inhibitor of nitrification (0.541). Nitrification inhibitor (Carboxymethyl Pyrazole, CMP) significantly reduced NO_3-N formation in soil up to 41 days after cotton planting (Ravi et al., 1999). The results showed that all the test nitrification inhibitors could significantly decrease the nitrate content in soil and pakchoi **-** chinese cabbage (*Brassica rapa*, subspecies *pekinensis* and *chinensis*) during whole growth stage, among which, DCD had the best effect, but the effect was differed on different soil types (Yu et al., 2006).

Similarly like in soil profile of 0.30-0.60 m, also in this case (0.60-0.90 m) the highest reduction of $N\text{-}NO_3^-/N_{min}$ ratio happened when urea containing both inhibitors (inhibitor of urease and inhibitor of nitrification) was applied into soil (table 5). Its application decreased $N\text{-}NO_3^-/N_{min}$ ratio by 15.6% comparing with application of urea without inhibitors (U). When urea containing only inhibitor of nitrification (U+IN) was applied the ratio of $N\text{-}NO_3^-/N_{min}$ was also considerably reduced by 13.9%. The results achieved by Jiao et al. (2004**)** showed that the treatments of inhibitors, especially the combined application of NBPT (N-(n-butyl) thiophosphoric triamide) and DCD, could increase soil NH_4^+-N by 2%-53%, inhibit NH_4^+ oxidation, decrease soil NO_3^--N concentration, increase soil total available N by 34%-44%, and increase wheat N uptake by 0.26%-6.79%. The best treatment was urease inhibitor (NBPT) combined with nitrification inhibitor (DCD) what is in accordance with our results.

The lowest effect of inhibitors was found out when urea with inhibitor of urease (U+IU) was applied. In this case lowering of $N\text{-}NO_3^-/N_{min}$ ratio represented 5.3% in comparison to pure urea application. Oppositely, application of LAD-27 fertilizer also in this soil profile significantly increased $N\text{-}NO_3^-/N_{min}$ ratio by 12.1% in comparison with urea non-containing inhibitors (table 5, table 6).

Average values of $N\text{-}NO_3^-/N_{min}$ ratio for four years duration of experiment, six terms of soil sampling, three soil depths and four repetions are illustrated in figure 1. This figure shows that in all three cases when urea containing inhibitor/inhibitors was applied it caused decrease of $N\text{-}NO_3^-/N_{min}$ ratio in soil in comparison to treatment U (urea without inhibitor). This decrease was represented by the following values: -11.7% (U+IN), -10.8% (U+IN+IU) and -9.4% (U+IU). Differences between the values of $N\text{-}NO_3^-/N_{min}$ ratio achieved in treatments fertilized with urea containing inhibitor/inhibitors were not statistically significant on the average of four years (figure 1).

On the other side application of LAD-27 fertilizer increased significantly this ratio by 23% in comparison to treatment fertilized with pure urea (treat. U).

The DCD inhibited remarkably the nitrification process for more then two months, maintaining significantly higher levels of ammonium in soil solution compared to normal urea (Montemurro et al., 1998).

Fertilization of wheat with urea without inhibitors as well as inhibitors increased grain yield significantly by 12.3 to 15.0% comparing to unfertilized control treatment (table 7). Comparing the grain yield of wheat in treatment fertilized by pure urea (U) with the yields achieved in treatments fertilized with urea containing inhibitors, one can see that an addition of inhibitor/inhibitors into urea increased grain yield insignificantly by 1.4% (treat. U+IN), 2.2% (treat. U+IU) and by 2.4% (treat. U+IN+IU).

The highest yield were achieved in treatments U+IU (7.35 t ha^{-1}) and U+IN+IU (7.36 t ha^{-1}). The differences of yields between respective fertilized treatments were not statistically significant (table 7). 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).

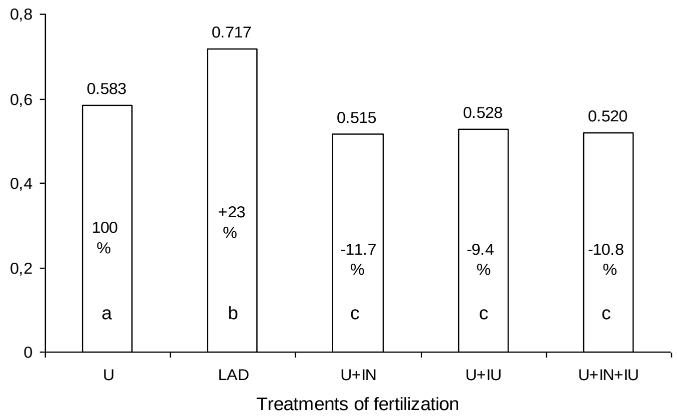

Figure 1 Ratio of $N\text{-}NO_3^-/N_{min}$ and decrease/increase of this ratio (%) in comparison to control (100%), average of years, sampling dates, depths and repetitions (LSD=0.053, α = 0.05)

Table 7

Effect of fertilizers with inhibitors on winter wheat grain yield

Treatment of nutrition	Grain yield (t ha^{-1}) Years 2009	2010	2011	2012	Average	Relatively (%) C=100 %	U=100 %
C	7.45	5.95	6.30	5.88	**6.40 a**	100.0	-
U	8.50	6.86	7.02	6.38	**7.19 b**	112.3	100.0
LAD	8.60	6.85	7.05	6.48	**7.25 b**	113.3	100.8
U + IN	8.55	6.94	7.15	6.53	**7.29 b**	113.9	101.4
U + IU	8.30	7.18	7.23	6.68	**7.35 b**	114.8	102.2
U+IN+ IU	8.27	7.18	7.26	6.71	**7.36 b**	115.0	102.4

LSD = 0.59; α = 0.05

Similarly like with wheat grain yields, the highest increments of grain yield were found out in treatment U+IN+IU (0.960 t ha^{-1}) and U+IU (0.953 t ha^{-1}). On the basis of grain yield increment and dose of nitrogen per hectare coefficient of natural effectiveness (K_{NE}) was calculated for respective treatments. As it can be seen from table 8, significantly higher values of coefficient of natural effectiveness (6.410-6.860) were achieved in treatments fertilized with inhibited urea (urea with inhibitor/s) than in treatment fertilized with pure urea (5.678) and LAD-27 (6.072). Statistically significant differences were not found out between treatments fertilized with inhibited urea (table 8).

Owing to the fact that urea with inhibitors was applied at one shot without splitting to partial doses during growing season (treat. U+IN; U+IN+IU) or by splitting to two partial doses (treat. U+IU) higher natural effectiveness of fertilization was achieved in these treatments as a consequence of better utilization of nitrogen in comparison to application of pure urea at which the same total dose of nitrogen was split to three partial doses (table 3). And again, also in this parameter, the best results, e.g. the highest coefficient of natural effectiveness was achieved in treatment fertilized with urea containing both inhibitors (6.860-tab. 8), that is there was created 6.860 kg of wheat grain per each kilogramme of applied fertilizer nitrogen in this treatment. 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).

Table 8

Natural effectiveness of winter wheat nitrogen fertilization

Treatment	Increment of grain yield (t ha^{-1})					Coeff. of natural effectiveness (K_{NE})				
	2009	2010	2011	2012	**Mean**	2009	2010	2011	2012	**Mean**
Control, no N	-	-	-	-	-	-	-	-	-	-
PIAGRAN 46	1.05	0.91	0.72	0.50	**0.795**	7.50	6.50	5.14	3.57	**5.678 a**
LAD 27	1.15	0.90	0.75	0.60	**0.850**	8.21	6.43	5.36	4.29	**6.072 a**
ALZON (U+IN)	1.10	0.99	0.85	0.65	**0.898**	7.86	7.07	6.07	4.64	**6.410 b**
PIAZUR (U+IU)	0.85	1.23	0.93	0.80	**0.953**	6.07	8.79	6.64	5.71	**6.803 b**
U+ IN+ IU	0.82	1.23	0.96	0.83	**0.960**	5.86	8.79	6.86	5.93	**6.860 b**

LSD = 0.51; α = 0.05
PIAGRAN 46 - normal urea, without inhibitors
ALZON 46 - urea containing inhibitor of nitrification
PIAZUR - urea + inhibitor of urease
U + IN + IU - urea + inhibitor of nitrification + inhibitor of urease
LAD 27 - ammonium nitrate with dolomite

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).

CONCLUSION

Achieved four –year average results indicate that application of inhibitors contained in urea significantly reduced $N\text{-}NO_3^-/ N_{min}$ ratio in soil by 11.7% (urea+inhibitor of nitrification), 10.8% (urea+both inhibitors) and 9.4% (urea+inhibitor of urease) in comparison to urea applied without inhibitors. Adverse effect of applied inhibitors on the winter wheat grain yield was not found out. Fertilization of wheat with urea without inhibitors as well as inhibitors increased grain yield significantly by 12.3 to 15.0% comparing to unfertilized control treatment. Howerer addition of inhibitor/inhibitors into pure urea increased grain yield insignificantly by 1.4-2.4%. These results also suggest that the both examined inhibitors contained in urea fertilizer have a potential to reduce portion of nitrates on mineral nitrogen content in soil. The highest coefficient of natural effectiveness of fertilization was achieved in treatment fertilized with urea containing both inhibitors (6.860), that is there was created 6.860 kg of wheat grain per each kilogramme of applied fertilizer nitrogen in this treatment. It was owing to the fact that urea with inhibitors was applied at one shot without splitting to partial doses during growing season in comparison to application of pure urea at which the same total dose of nitrogen was split to three partial doses. Both urease inhibitor and nitrification inhibitor can be tool to manage N loss profitably in today's economic climate.

Table 9

Content of nitrate nitrogen and ammonium nitrogen in soil ($mg.kg^{-1}$)
average of 4 experimental years

Date of Sampling	Depth (m)	U		LAD		U + IN		U + IU		U+IN+IU	
		$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$
		mg kg^{-1}									
25.3.	0.-0.3	4.4	8.9	4.4	8.9	4.4	8.9	4.4	8.9	4.4	8.9
	0.3-0.6	2.9	6.3	2.9	6.3	2.9	6.3	2.9	6.3	2.9	6.3
	0.6-0.9	2.8	6.4	2.8	6.4	2.8	6.4	2.8	6.4	2.8	6.4
17.4.	0.0-0.3	16.5	7.5	12.3	20.4	40.5	9.4	20.4	7.5	25.7	8.8
	0.3-0.6	13.0	5.7	8.6	13.3	14.5	7.8	12.2	7.0	18.4	8.3
	0.6-0.9	5.9	5.7	6.8	9.4	16.9	6.2	10.3	7.2	16.7	7.0
10.5.	0.0-0.3	11.2	6.0	16.7	59.2	28.1	9.4	43.9	7.3	83.3	9.6
	0.3-0.6	6.3	4.8	5.3	14.7	8.7	6.3	12.0	5.0	18.8	7.2
	0.6-0.9	4.3	6.1	4.0	10.7	9.7	6.3	5.1	5.2	6.2	5.3
7.6.	0.0-0.3	7.9	27.1	5.7	16.7	7.8	8.6	9.2	4.6	24.1	24.8
	0.3-0.6	3.9	6.7	4.5	9.5	5.7	7.1	5.4	7.5	7.9	11.2
	0.6-0.9	3.3	4.3	3.2	8.0	4.4	5.1	4.6	5.8	3.8	6.4
4.7.	0.0-0.3	4.4	10.4	3.3	8.1	6.1	8.6	10.7	2.5	5.6	25.3
	0.3-0.6	3.1	6.8	2.5	9.6	3.3	9.7	4.5	8.2	4.0	18.4
	0.6-0.9	2.4	6.8	3.4	8.6	3.0	8.4	3.3	9.9	3.6	8.1
23.7	0.0-0.3	2.7	6.4	2.2	6.4	3.2	5.7	7.3	9.7	5.1	13.9
	0.3-0.6	1.9	4.8	1.7	7.3	2.2	6.8	4.5	3.3	4.1	10.8
	0.6-0.9	1.7	5.0	2.0	6.9	1.9	6.4	2.8	8.1	4.1	5.7

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