EFFECT OF UREASE AND NITRIFICATION INHIBITORS ON WHEAT YIELD

MUHAMMAD ARSHAD KHAN[1], ZAHIR SHAH[1]*, ABDUR RAB[3],
MUHAMMAD ARIF[4] and TAHIR SHAH[2]

1 *Department of Soil and Environmental Sciences, The University of Agriculture, Peshawar, Pakistan*
2 *Department of Animal Health, Faculty of Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan*
3 *Department of Horticulture, The University of Agriculture, Peshawar – Pakistan*
4 *Department of Agronomy, The University of Agriculture, Peshawar - Pakistan*
**Correspondence author: zahirshah@aup.edu.pk*

ABSTRACT

A field experiment was conducted to assess the effect of urease and nitrification inhibitors on wheat yield at the Research Farm of the University of Agriculture Peshawar during 2011-12. The experiment was arranged in a randomized complete block design with 3 replications. Urea coated with urease inhibitor (agrotain) or urease combined with nitrification inhibitors (super urea) was applied each at 60 kg and 120 kg N ha^{-1} in 2 or 3 splits. The results showed that the inhibitors treated urea significantly increased the yield and yield components of wheat over the non-inhibitor urea treatment. The results further showed that super urea was more effective than agrotain in terms of increased yield. The improvement in yield with inhibitors was more with low (60 kg N ha^{-1}) than with high dose of urea (120 kg N ha^{-1}). Application of urea in 3 splits was better than 2 splits. The highest improvement of 42.9% in grain yield and 45.1% in total N uptake was obtained in treatment receiving super urea at 60 kg N ha^{-1} compared with non-inhibitor treated urea at 60 kg N ha^{-1}. The 2nd highest improvement of 37.5% in grain yield and 38.0% in total N uptake was obtained in treatment receiving agrotain (urease inhibitor) treated urea at 60 kg N ha^{-1} compared with non-inhibitor treated urea at 60 kg N ha^{-1}. These results suggested that the use of N inhibitors significantly increased the yield of wheat. However, the combined use of urease and nitrification inhibitors (super-urea) was better than urease inhibitor alone (agrotain). Based on the findings of this study, improvement in wheat yield caused by urease and nitrification inhibitors is likely due to increased N use efficiency.

Keywords: ***Agrotain, nitrification inhibitor, NUE, super-urea, wheat yield, urea, urease inhibitor***

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INTRODUCTION

The soils of Pakistan are deficient in nitrogen (Shah *et al*., 2012) and the farmers are using nitrogenous fertilizers, mainly urea, to remove its deficiency as crops respond strongly to these fertilizers (Ehsanullah *et al*., 2012; Ali and Noorka, 2013). However, inefficient use of N fertilizers may add to environmental degradation, especially in intensive agricultural systems where the recovery or nutrient use efficiency by crops is comparatively low. Nitrogen (N) use efficiency for cereal production worldwide is only 33% (Raun and Johnson, 1999). Nitrogen fertilizer which is extensively used in world agriculture is urea. Urea when applied to soil is hydrolyzed by urease enzyme to NH_3 and CO_2 with an increase in pH and NH_4^+ accumulation in soil. Losses of gaseous NH_3 can take place with surface application of urea and may approaches up to 50% of the N fertilizer applied (Terman, 1979; Catchpoole, 1975), particularly in alkaline calcareous soil, soil with low buffering capacity or soils with high organic carbon contents (Fenn and Hossner, 1985). Nitrogen losses from urea can be minimized through the reduction of urease activity which will further delay urea hydrolysis. Similarly, urea fertilizer is subject to NH_3 volatilization through the activity of urease enzyme which is found in soils all over the world. There are many products that have been developed to delay urea hydrolysis or other N transformation processes to synchronize availability of N with the plant needs. The important products include phenyl phosphorodiamidate (PPD), hydroquinone (HQ), N-(n-butyl) thiophosphorictriamide (NBPT), phenyl mercuric acetate (PMA), catechol and others as urease inhibitors and 3,4-dimethyl-1H-pyrazoliumdihydrogen (DMPP), dicyandiamide (DCD), thiosulphate, neem, karanjin, nitrapyrine (N-serve) and others as nitrification inhibitors.

Agrotain, which is available in market since 1996,when added to urea-based N products, slows down the activity of urease enzyme and slows the conversion of NH_4^+ to gaseous NH_3 reducing volatilization loss of N (Watson *et al*., 1990). The active compound in agrotain is NBPT which blocks the urease enzyme from its binding sites and preventing its reaction with urea (Manunza *et al*., 1999). The NBPT has got the potential to minimize N losses at low concentration and can increase production of various crops (Watson *et al*., 2008; Dawar *et al*., 2011). Agrotain can block hydrolysis of urea up to 14 days after its application. Among the several urease inhibitors, N-(n-butyl) thiophosphorictriamide (NBPT; Beyrouty *et al*., 1988), is very efficient and reduces urea hydrolysis and volatilization losses in various soils. It has been proved that agrotain application reduced NH_3 emissions over urine alone by 29% in autumn, 93% in spring and 31% in summer, however, had little effect on N_2O emission (Zaman *et al*., 2009).

Nitrification inhibitor (NI) application is also considered as an approach to reduce losses from N fertilizers and to increase N use efficiency (Prasad and Power, 1995). Since nitrification inhibitors influence the conversion of ammonium to nitrate, they can only be used efficiently on fertilizer products that contain or convert to ammonium form. These include anhydrous ammonia, ammonium sulfate, urea ammonium nitrate and liquid manure. A number of nitrification inhibitors have been developed to inhibit the process of nitrification. Among nitrification inhibitors, nitrapyrin (2-chloro-6-[trichloromethyl] pyridine) has been commercially used since late-1960's. Randall *et al*.(2003) and Randall and Vetsch (2005) in Minnesota reported that higher corn yield was obtained with nitrapyrin in fall-applied N, however, greatest N-use efficiency was obtained with spring-applied N. Dicyandiamide (DCD) is another nitrification inhibitor and is shown effective in slowing nitrification process; potentially increase autumn forage production and N uptake when DCD was applied in combination with urea (Srinivas *et al*.,1999).

Super urea (SU, Agrotain International, St. Louis, Missouri, USA) is a kind of nitrogenousfertilizer that has been treated with NBPT and dicyandiamide. This granular fertilizer is blue in color and contains both N-(n-butyl) thiophosphorictriamide, a urease inhibitor which reduces NH_3 volatilization from urea, and DCD, a nitrification inhibitor which slows down nitrification process. Combination of NBPT and DCD is believed to allow complete utilization of N by crops. It is used in both mass blends and solutions as a nitrogen source. Similarly, it blends well with other fertilizer materials to produce a variety of nitrogen, phosphorus and potassium grades and is soluble in water (about 20% N). Higher yield was achieved with urea treated with agrotain and super urea as compared to urea alone or when urea with several additives was applied in side-dress (Schwab and Murdock, 2010). The combination of urea with urease and nitrification inhibitor (^{15}N-U + NBPT + DCD) can retard hydrolysis of urea, increase wheat yield and protein content by 27.77% and 10.19% respectively (Hou *et al.,* 2006). It also increase ^{15}N uptake by wheat and reduce the loss of urea-N in soil-wheat system.

For increasing N-use efficiency, one must minimize the N losses and increase its consumption by the crop. The combined application of hydroquinone (HQ) and dicyandiamide (DCD) inhibitors to spring wheat had minimized gaseous N losses compared to HQ or DCD alone which showed that the combination of HQ and DCD is an efficient way to increase N use efficiency, quality of wheat crop and ultimately reduces nitrogen losses from the soil environment (Xu *et al*., 2000). Similarly, the application of a urease inhibitor, HQ, and a nitrification inhibitor, DCD, together with urea (U) to rice crop grown in pots significantly reduced emission of N_2O and CH_4 as compared with the treatment whereby urea was applied alone (Xu *et al*., 2002). In an earlier study by Chen *et al.* (1998) urea combined with HQ and DCD (U + HQ + DCD) have improved grain yield by 32% and N recovery by 69 % in spring wheat as compared to urea alone. Therefore, urea in combination with NBPT and NBPT + DCD can increase N recovery by crops by minimizing ammonia volatilization and thus increases nitrogen use efficiency.

The soils of Pakistan are mostly alkaline and calcareous in nature and are conducive to N losses via NH_3 volatilization. However, limited work has been done in this of the world to assess the impact of use of urease and nitrification inhibitors with urea fertilizer on minimizing N losses and increasing crop yields. This study was therefore undertaken to investigate the effect of use of urease and nitrification inhibitors with urea fertilizer on yield and N use efficiency and also to compare the effect of two and three split applications of urea on N recovery and yield of wheat crop.

MATERIALS AND METHODS

A field experiment was conducted at the Research Farm of the University of Agriculture Peshawar to study the effect of urease and nitrification inhibitors on wheat yield during 2011-12. Soil of the experimental site was silt loam,alkaline in reaction (pH 8.06), non- saline (EC 0.16 dSm^{-1}), strongly calcareous (lime 16.0%) and low in organic matter (< 1%) Table 1. The treatments were 1) control (no urea), 2) urea at 120 kg N ha^{-1} (in two splits), 3) urea at 60 kg N ha^{-1} (in two splits), 4) urease inhibitor (agrotain) coated urea at 120 kg N ha^{-1} (in two splits), 5) urease inhibitor (agrotain) coated urea at 60 kg N ha^{-1} (in two splits), 6) urease plus nitrification inhibitor coated urea (super urea) at 120 kg N ha^{-1} (in two splits), 7) urease and nitrification inhibitors coated urea (super urea) at 60 kg N ha^{-1} (in two splits), 8) urease and nitrification inhibitors coated urea (super urea) at 120 kg N ha^{-1} (in three splits), 9) urea at 120 kg N ha^{-1} (in three splits) and 10) urea at 60 kg N ha^{-1} (in three splits).

Table 1. ***Some important soil characteristics of the experimental site***

Property	Unit	Value
Clay	%	9.10
Sand	%	14.22
Silt	%	76.68
Textural class	-	Silt loam
$pH_{(se)}$	-	8.06
$EC_{(se)}$	dSm^{-1}	0.16
Lime	%	16.00
Organic matter	%	0.89

Agrotain product was used as urease inhibitor while super-urea was used as urease and nitrification inhibitors. Experimental design was randomized complete block and each treatment was replicated three times. Each treatment plot was 5 m wide and 3 m long with 0.5 m distance between the treatments and 1 m distance between the blocks. All treatment plots received P_20_5 at 90 kg ha^{-1} and K_2O at 60 kg ha^{-1} as basal dose at sowing time. Nitrogen was applied either in two splits or three splits as per experimental plan. Urea either in two or three splits was applied both with and without inhibitors per experimental plan. The crop was sown in November 2011 and harvested in May 2013. Recommended cultural practices were followed throughout the growing period. The crop was irrigated with canal water when needed. The crop was harvested at maturity and data was recorded on biological yield, grain yield, straw yield, number of spikes m^{-2}, number of grains per spike, 1000-grain weight and total N uptake in crop.

Laboratory Analysis

Total mineral N in soil samples was determined by the steam distillation method as given in Mulvaney (1996). Total N in soil and plant samples was determined by the Kjeldhal method as described in Bremner (1996). Soil pH and EC weredetermined in the saturated soil extract. Soil extract was read for pH on pH meter (Thomas, 1996) and for EC on EC meter (Rhoades, 1996). Organic matter in soil samples was determined by the Walkley-Black procedure as described in Nelson and Sommers (1996). The amount of lime in soil samples was determined by the acid neutralization method as described in U.S. Salinity Laboratory Staff (1954). Texture of soil sample was determined by the bouyoucos hydrometer method (Gee and Bauder, 1986).

Statistical Analysis

The data recorded were analyzed statistically by the methods described in Steel and Torrie (1980) using M Stat C package. LSD test was applied for means comparison between treatments.

RESULTS AND DISCUSSION

The results obtained on the effect of urease and nitrification inhibitors on yield and N uptake of wheat are presented and discussed as under:

Biological Yield

The results obtained on biological yield of wheat as influenced by N inhibitors treated and untreated urea treatments are presented in Table 2. The results showed that on average the maximum biological yield of 11743 kg ha^{-1} was obtained in the treatment which had received super-urea (urease plus nitrification inhibitors treated urea) at the rate of 120 kg N ha^{-1}. The super-urea improved the biological yield by 30.2% with 60 kg N and 25.0% with 120 kg N ha^{-1}, whereas agrotain (urease inhibitor treated urea) improved biological yield by 25.2% with 60 kg N and 17.4% with 120 kg N ha^{-1} Table 2. Zaman *et al*. (2009) also showed that agrotain and dicyandiamide (DCD) in combination were more effective in minimizing NH_3 losses and N_2O emission, and in controlling urea hydrolysis, improving pasture production and retaining N in NH_4^+ form. Other researchers also observed similar results whereas the combined use of nitrification and urease inhibitors reported better results in terms of increasing crop yields than their use alone (Dawar *et al*., 2010; Nasima *et al*., 2010).

Table 2. ***Percent increase in biological yield of wheat by urease (Agrotain) or urease plus nitrification (Super-urea) inhibitors treatments over non-inhibitors treatments***

Treatments	Biological yield (kg ha^{-1})	Increase by inhibitors (kg ha^{-1})	% Increase by inhibitors	% increase by super urea over agrotain
Commercial urea @ 60 kg N ha^{-1}	7231	-	-	-
Agrotain treated urea @ 60 kg N ha^{-1}	9668	2437	25.2	-
Super urea @ 60 kg N ha^{-1}	10365	3134	30.2	6.72
Commercial urea @ 120 kg N ha^{-1}	8806	-	-	-
Agrotain treated urea @ 120 kg N ha^{-1}	10666	1860	17.4	-
Super urea @ 120 kg N ha^{-1}	11743	2937	25.0	9.17

Grain Yield

The results obtained on grain yield of wheat showed that on average the highest grain yield of 5282 kg ha^{-1} was obtained in the treatment receiving super-urea (urease plus nitrification inhibitor treated urea) at 120 kg N ha^{-1} followed by 4942 kg N ha^{-1} obtained in the treatment receiving agrotain (urease inhibitor treated urea) at 120 kg N ha^{-1} Table 3. Super urea improved the grain yield of wheat by 42.9% with 60 kg N and 27.6% with 120 kg N ha^{-1} while agrotain improved the grain yield by 37.5% with 60 kg N and 22.6% with 120 kg N ha^{-1} as compared to treatments receiving untreated urea at 60 or 120 kg N ha^{-1} Table 3. These results are consistent with the findings of Hou *et al*. (2006) who reported that combination of urea (^{15}N-U) with urease inhibitors, N-(n-butyl) thiophosphorictriamide (NBPT), and a nitrification inhibitor, dicyandiamide (DCD), significantly reduced urea hydrolysis and in turn increased grain yield of wheat by 27.8%.

Table 3. ***Percent increase in grain yield of wheat by urease (Agrotain) or urease plus nitrification (Super-urea) inhibitors treatments over non-inhibitors treatments***

Treatments	Grain yield (kg ha^{-1})	Increase by inhibitors (kg ha^{-1})	% Increase by inhibitors	% increase by super urea over agrotain
Simple urea @ 60 kg N ha^{-1}	2794	-	-	-
Agrotain treated urea @ 60 kg N ha^{-1}	4470	1676	37.5	-
Super urea @ 60 kg N ha^{-1}	4897	2103	42.9	8.71
Simple urea @ 120 kg N ha^{-1}	3826	-	-	-
Agrotain treated urea @ 120 kg N ha^{-1}	4942	1115	22.6	-
Super urea @ 120 kg N ha^{-1}	5282	1455	27.6	6.43

Other Yield Components

The results obtained on straw yield of wheat revealed that maximum straw yield of 6798 kg ha^{-1} was obtained in the treatment receiving super urea (urease plus nitrification inhibitors treated urea) at 120 kg N ha^{-1} in three splits and this was statistically at par with those obtained with super urea at 120 kg N ha^{-1} in two splits (6467 kg ha^{-1}) or agrotain (urease inhibitor treated urea) at 120 kg N ha^{-1} (5768 kg ha^{-1}) Table 4. The minimum straw yield of 3684 kg ha^{-1} was obtained in the control treatment. The results showed that straw yield of wheat increased with increasing level of N. Furthermore, the straw yield of wheat was generally greater for three than for two splits of urea application.

The results obtained on number of spikes as influenced by N inhibitors and split application of urea revealed that the significantly ($p<0.05$) highest number of spikes (233 spikes m^{-2}) were obtained for treatment receiving super-urea (urease plus nitrification inhibitor treated urea) at 120 kg N ha^{-1} in three splits Table 4. The next significantly ($p<0.05$) highest number of spikes (222 spikes m^{-2}) were obtained for treatment receiving super urea at 120 kg N ha^{-1} in two splits. The lowest number of spikes m^{-2} (153) were obtained in the control treatment and this was significantly lowest than all fertilizer treatments. Moreover, the number of spikes increased with increasing level and splits of urea application.

Table 4. ***Effect of urease inhibitor (Agrotain) and urease plus nitrification inhibitor (Super-urea) on yield and yield components of wheat***

Treatment	N rate ($kg\ ha^{-1}$)	Straw yield ($kg\ ha^{-1}$)	No. of spikes (m^{-2})	No. of grains $spike^{-1}$	1000-grain weight (g)
Control	0	3684e	153g	36g	35.5g
Simple urea (2 splits)	120	4980cd	190e	46e	42.4e
Simple urea (2 splits)	60	4437ef	181f	41f	39.1f
**Agrotain treated urea (2 splits)	120	5768abc	211c	54bc	51.2c
Agrotain treated urea (2 splits)	60	5197cd	198d	52cd	50.0c
***Super urea (2 splits)	120	6467ab	222b	57ab	54.6b
Super urea (2 splits)	60	5423bcd	210c	54bc	53.1b
Super urea (3 splits)	120	6798a	233a	59a	57.0a
Simple urea (3 splits)	120	5668bcd	199d	50d	47.1d
Simple urea (3 splits)	60	4689de	187e	46e	43.7e
LSD (0.05)		1051	4.44	2.82	1.57

**Means followed by same letter(s) within columns are statistically non-significant (P<0.05).*
***Agrotain contain urease inhibitor; ***Super urea contain both urease and nitrification inhibitor.*

The significantly ($p<0.05$) highest number of grains $spike^{-1}$ (59 grain per spike) were obtained for treatment receiving super urea (urease plus nitrification inhibitor treated urea) at 120 kg N ha^{-1} in three splits. This was however statistically at par with that (57 grains per spike) obtained with super urea at 120 kg N ha^{-1} in two splits. The lowest number of grains per spike (36 grains per spike) were obtained in the control treatment and this was significantly ($p<0.05$) lowest than all fertilizer treatments.

As with other yield components, the significantly ($p<0.05$) highest 1000-grain weight of 57 g was obtained for treatment receiving super urea (urease plus nitrification inhibitors treated urea) at 120 kg N ha^{-1} in three splits Table 4. The lowest 1000-grain weight of 35.5 g was obtained in the control treatment and this significantly ($p<0.05$) lowest than all fertilizer treatments. It was however noticed that 1000-grain weight was generally increased with increasing level and number of split application of urea. Almost similar results have been reported for the influence of urease and nitrification inhibitors on yield and yield components of wheat and other crops (Xu *et al*., 2000; Xu *et al*., 2002; Hou *et al*., 2006; Zaman *et al*., 2009).

Nitrogen Uptake

On average, the highest N uptake of 108.9 kg ha^{-1} was obtained in treatment receiving super-urea (urease plus nitrification inhibitor treated urea) at 120 kg N ha^{-1} followed by 104.0 kg ha^{-1} in treatment receiving super urea at 60 kg N ha^{-1} Table 5. The super-urea increased the N uptake of wheat by 45.1 % with 60 kg N and none with 120 kg N ha^{-1}. However, agrotain (urease inhibitor treated urea) increased N uptake of wheat by 38.0 % with 60 kg N and 29.2 % with 120 kg N ha^{-1}. The results also showed that N uptake increased with increasing the number of split application of urea both at 120 and 60 kg N ha^{-1} (data not shown here). Chen *et al*. (1998) reported that urea in combination with dicyandiamide plus hydroquinone (U + DCD + HQ) recovered 69% of urea-N in plant and 73 % of the recovered N was transformed into grain protein by spring wheat. Other workers also reported that the use of urease and nitrification inhibitors reduced N losses and increased N use efficiency by various crops (Xu *et al*., 2000; Xu *et al*., 2002; Zaman *et al*., 2009).

Table 5. ***Percent increase in N uptake of wheat by urease (Agrotain) or urease plus nitrification inhibitors (Super-urea) treatments over non-inhibitors treatments***

Treatments	Total N uptake (kg ha-1)	Increase by inhibitors ($kg\ ha^{-1}$)	Increase by inhibitors (%)	Increase by super-urea over agrotain (%)
60 kg N without inhibitors (2 splits)	57.1	-	-	-
60 kg N with agrotain inhibitor (2 splits)	92.3	35.1	38.0	-
60 kg N with super-urea inhibitor (2 splits)	104.0	46.9	45.1	11.25
120 kg N without inhibitors (2 splits)	77.1	-	-	-
120 kg N with agrotain inhibitor (2 splits)	108.9	31.8	29.2	-

These results revealed that the use of nitrification inhibitors with urea did improve the yield and yield components of wheat planted on a calcareous alkaline soil. Using both urease and nitrification inhibitors with urea performed much better than the use of urease inhibitor alone. Thus it could be concluded that strongly calcareous alkaline soils are highly conducive to rapid urea hydrolysis and nitrification processes which lead to N losses. Curtailing such processes with urease or nitrification inhibitors prolong the existence of nitrogen in soil giving greater opportunity to plants for its utilization and hence reducing chances of its volatilization. These results therefore suggest that the use of urea on highly calcareous alkaline soils need special management to reduce its loss and maximize its efficiency or plant utilization. The use of nitrogen inhibitors seems one of the promising strategies to reduce N losses from urea on calcareous alkaline soils provided other conditions are favorable such as soil organic matter and temperature (Ali *et al*., 2012).

CONCLUSIONS AND RECOMMENDATIONS

The N inhibitors treated urea performed better than untreated urea in increasing the yield and yield components of wheat. Among inhibitors, the urease + nitrification inhibitors (super-urea) was superior to urease inhibitor alone (agrotain) in increasing the yield and yield components of wheat. Both inhibitors increased N uptake compared with non-inhibitor treated urea. Super urea performed better than agrotain in terms of increasing N use efficiency. The use of inhibitors with low (60 kg N ha^{-1}) level of urea was better than with high (120 kg N ha^{-1}) level of urea. The application of urea in three splits was better than two splits. There seems potential benefit of the combined use of urease and nitrification inhibitors (super-urea) than urease inhibitor alone (agrotain). Thus, the use of N inhibitors with urea is recommended for increasing crop production and N use efficiency on a calcareous alkaline soil. However, economics of the use of inhibitors must be worked out carefully before it is recommended to farmers.

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