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NEEM (*Azadirachta indica*) OIL COATED UREA IMPROVES NITROGEN USE EFFICIENCY AND MAIZE GROWTH IN AN ALKALINE CALCAREOUS SOIL

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Two independent experiments were conducted to assess neem oil coated urea (NOCU), as a natural nitrification inhibitor and to improve nitrogen use efficiency in maize. In first experiment, soil incubation with increasing levels (i.e. 1%, 2% and 3% w/v) of neem oil coating was done for 28 days to monitor the release of NH_4^+ and NO_3^-contents. Neem oil coated urea slowed down the release of mineral N with maximum nitrification inhibition (40%) at 3% level of neem coating as compared to 2% and 1%, hence selected for next study. In the subsequent pot study, NOCU was applied according to recommended dose (250 kg ha^{-1}) of urea at three different levels (50%, 75% and 100%) for maize crop. There was a significant increase in N contents of shoot and grains that ultimately provoked high protein contents in maize grains. Application of NOCU enhanced the N uptake due to its increased availability for longer period of time, resulting in higher grain yield. Maximum agronomic efficiency (24.38 g g^{-1}) of applied N was observed with 75% dose of NOCU, while maximum recovery efficiency (0.44 g g^{-1}) was recorded with 100% dose of NOCU. Conclusively, application of NOCU saves 25% N application compared to ordinary urea.
Keywords: Nitrification inhibition, ammonium, nitrate, agronomic efficiency; recovery efficiency.

INTRODUCTION

Environmental losses of nitrogen (N) are the primary reason for low use efficiency of nitrogenous fertilizers (Godfrey *et al.,* 2010; Hawkesford, 2014). Availability of N, its uptake and translocation within plant affect physiological linked phenomenon that deal with biomass development and grain production(Below *et al.,* 1985; Kaizzi *et al.,* 2012).Nitrogen plays a significant role in plant growth and development, as it is a structural component of chlorophyll, proteins and enzymes (Haque *et al.,* 2001).. Photosynthetic processes, net assimilation rate and leaf area duration increases with optimum supply of N (Ahmad *et al.,* 2009), which contributes elevated grain output (Cheema *et al.,* 2001; Tsialtas and Maslaris, 2008; Rafiq *et al.,* 2010). To fulfill the food requirement of increasing population, N is provided through synthetic fertilizers and mostly it is added in the form of urea which is subjected to numerous losses due to rapid hydrolysis. Most of agricultural soils in Pakistan are deficient in N due to high temperature, causing decomposition of organic matter (Shah *et al.,* 2012).

Urea (46% N) as an important N fertilizer used globally because of its storage features and cost effectiveness. Unfortunately, in Pakistan up to 70% of the urea is lost into environment and becomes unavailable to plants. Nitrogen losses through ammonia volatilization, nitrification and denitrification adversely affect ecosystem, as nitrogen per oxide is a greenhouse gas that is partially responsible for global warming. Beyond that, nitrate buildup in lakes causes eutrophication and elevated concentration > 10ppm causes serious health hazards for animals and humans (Dinnes *et al.,* 2002). In order to decrease environmental and soil pollution, there is need to decrease rapid hydrolysis of urea, prolonging N availability for optimum plant growth, development and to improve fertilizer use efficiency. Application of nitrification inhibitors (NIs) is a beneficial approach to minimize N losses and for limiting environmental threats caused by nitrogenous fertilizers (Subbarao *et al.,* 2006; Prasad, 2009). These compounds delay bacterial oxidation of nitrogen at first step of conversion of ammonium to nitrate by impeding the nitrobacteria activities. Resultantly, N is slowly released from fertilizers which reduces its loss and enhances NUE (nitrogen use efficiency) (Motavalli *et al.,* 2008). Mainly, NO_3^- inhibition occurs by restricting the activities of ammonia monooxygenase (AMO) enzyme which is a part of *Nitrosomonas* bacteria (Subbarao *et al.,* 2006). These inhibitors stabilize the NH_4^+ contents to slow down the nitrification process and increase the availability of nitrogen to plants in the form of NH_4^+ (Singh and Verma, 2007). Furthermore, NIs reduce the N_2O emission and NO_3^- leaching (Kumar *et al.,* 2000).

Some natural plant materials possess the properties of nitrification inhibition and slow down the urea hydrolysis rate. Plant based NIs, being biodegradable and eco-friendly, hold considerable promise to conserve natural nitrogen pool and improve N prestige by promoting of low NO_3^- ecosystems (Subbarao *et al.,* 2006; Lata *et al.,* 2004). Several studies have revealed nitrification process inhibition ability is also present naturally in some plants and their byproducts (Kiranand Patra**,** 2002; Kiran *et al.,* 2003; Lata *et al.,* 2004; Patra *et al.*, 2006 and 2009). Rendle (*Brachiaria humidicola***),** karanj **(***Pongamia glabra* Vent.), neem **(***Azadirachta indica***),** sweet sorghum (*Sorghum bicolor* L.), linseed oil (*Linum usitatissimum* L.), tea (*Camellia sinensis* L.), mahuwa (*Madhuca latifolia*), mint (*Mentha spicata* L.), *Artemisia annua* L., *Pyrethrum* species and natural essential oils are tested sources of natural nitrification inhibition. Neem and its products (oil and cake) are claimed to have strong nitrification inhibition qualities (Prasad *et al.*, 1995; Patra *et al.,* 2006).Its relatively low cost and ubiquitous availability in south Asia, promoted testing for improving NUE of a high value and N intensive crop, such as maize. Hence, the research was designed to decrease the rapid hydrolysis of urea and prolong N availability to maize by slowing down the process of nitrification, which may increase NUE.

MATERIALS AND METHODS

1st Experiment*:** An incubation study was conducted for 28 days under controlled conditions. 250 g of soil was filled in plastic container. Nitrogen was applied @ 200 mg kg^{-1} and incubated at 25 C^{o}. Moisture was maintained at field capacity throughout study. Sampling of soil was done after 3, 7, 14, 21 and 28 days of study for NO3-N and NH_4^+-N determination. Neem **(Azadirachta indica***)** oil was used as nitrification inhibitor. Experiment was run under CRD **(**completely randomized design**)** and with three replicates. The treatments consist of; T_1 = Control (No Urea, T_2 = Ordinary urea, T_3 = Urea with 3% Neem oil coating, T_4 = Urea with 2% Neem oil coating, T_5 = Urea with 1% Neem oil coating.

***Coating of urea*:** The coating of urea was done using neem oil extracted from seeds of neem at the rate of 1%, 2% and 3% on w/v basis. Oil was extracted with acetone after pressing the seeds through cold-process (Nicoletti *et al.,* 2012). After extraction, oil was directly sprayed onto urea for homogenous coating. After coating, the fertilizer was spread on plastic sheet containing very thin layer of plaster of Paris so that granules do not stick each other. The coated urea was dried under shade and packed in moisture free bags.

***Soil inorganic elemental nitrogen determination (mg kg^{-1})*:** Potassium sulfate extraction method of Clare and Stevenson (1964) through idophenol blue method was used for ammonical contents determination in soil by taking reading on spectrophotometer at 660nm aborbance (UV-1201, Shimadzu, Tokyo, Japan).While DTPA extractable method with ammonium bicarbonate was invented by Soltanpour and Schwab (1977) for determination of nitrate–nitrogen in soil, which is proceeded later on through reduction with hydrazine and absorbance reading at540-nm wavelength (Kamphake *et al.,* 1967) was taken.

***Nitrification inhibition (%)*:** To calculate total inhibition through nitrification in percentage, Sahrawat (1980) method was used.

$$\% \text{ Inhibition of nitrification} = \frac{(C\text{- } S) \times 100}{C}$$

Where S= concentration of NO_3^-–N in urea treated soil, C= concentration of NO_3^-–N in urea treated soil with test compound i.e. NIs.

***2nd Experiment*:** A pot study was conducted in glass house of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad (UAF), Pakistan to improve production, physiology and efficient uptake and utilization of nitrogen in maize using 3% neem oil coated urea on w/v basis. Soil was air dried, ground, and after passing through a 2 mm sieve, it was mixed thoroughly and the pots were filled with 12 Kg soil. Maize variety Monsanto, 6614 was tested in this study. In Table 1, basic soil physically and chemically analyzed parameters are given. Statistical design of CRD with three replicates and five treatments was used in pots. Nitrogenous and phosphorus fertilizers (Urea 46 % N), diammonium phosphate (DAP 18 % N and 46 % P), while for potassium, sulfate of potash (SOP 50% K) fertilizers were used according to farmer practice of 250-160-120 kg/ha. Five seeds per pot were planted and one plant was maintained after 10 days of germination. Moisture level in the pots was maintained according to requirement of plants through distilled water.

Table 1. Physico-chemical properties of soil used for experiments.

Parameter	Incubation Experiment	Pot Experiment
Textural class	Sandy clay Loam	Sandy clay Loam
pH_s	8.1	8.1
EC_e	1.16 dS m^{-1}	1.67 dS m^{-1}
Cation exchange capacity	14.5 $cmol_c$ kg^{-1}	16.3 $cmol_c$ kg^{-1}
Soluble Ca^{2+} + Mg^{2+}	9.0 $mmol_cL^{-1}$	11.0 $mmol_cL^{-1}$
Soluble Na^+	16.68 $mmol_cL^{-1}$	19.57 $mmol_cL^{-1}$
Extractable K^+	86 mg kg^{-1}	76 mg kg^{-1}
Total Nitrogen	0.03 %	0.04 %
NO_3^--N	3.8 mg kg^{-1}	3.2 mg kg^{-1}
NH_4^+-N	2.71 mg kg^{-1}	2.47 mg kg^{-1}
Available Phosphorus	7.34 mg kg^{-1}	6.41 mg kg^{-1}
Organic matter	0.64 %	0.57 %
Saturation percentage	37 %	39 %

***Growth and Yield measurements*:** At maturity, maize was harvested and data regarding plant height was measured with meter rod and dry matter produced was recorded by drying shoot and root in the oven till constant weight. The productive yield parameter related to 100 grains weight was also recorded.

***Physiological parameters*:** The physiological parameters were recorded after 40 days of germination. CIRAS-3 was used for recording physiological parameters regarding photosynthetic rate, transpiration rate and photosynthetic water use efficiency (PP System, Amesbury, MA, USA). A flux of 1000 µmol $m^{-2}s^{-1}$ photon was used to provide light to cuvette through Light emitting diodes (LED). To calculate these parameters two extended leaves from each experimental unit were selected. Photosynthetic to transpiration rate ratio was used to calculate water use efficiency.

Water use efficiency (WUE) =Photosynthetic rate (A)/ Transpiration rate (E)

***Chlorophyll contents*:** SPAD-502 meter (Konica-Minolta, Japan) was used for estimation of chlorophyll contents (SPAD Value) in plant leaves.

***Shoot and Grain Nitrogen (%)*:** Plant samples were finely ground with a Wiley mill fitted with stainless steel chamber and blades. Wet digestion of plant samples was done through the protocol of Wolf (1982). Deionized water was used later to make final volume of digested samples. Nitrogen contents were determined with Kjeldhal method (Jackson, 1962). The protein percentage was measured by multiplying nitrogen concentration with conversion factor 6.25.

***Nitrogen uptake*:** Nitrogen uptake was computed by formula described below

$$\text{N Uptake} = \frac{\text{Concentration of N (\%)} \times \text{Dry Matter (g pot}^{-1})}{100}$$

***Nitrogen use efficiency*:** Nitrogen use efficiency indices were determined as described by Cassman *et al*. (2002).

Agronomic efficiency (AEN) (g g^{-1})

$$\text{AEN} = \frac{(YN - Y0)}{FN}$$

Recovery efficiency (REN) (g g^{-1})

$$\text{REN} = \frac{(UN - U0)}{FN}$$

FN – amount of N fertilizer applied (g pot^{-1}); YN – crop yield with applied N(g pot^{-1}); Y0 – crop yield (g pot^{-1}) in a control treatment without addition of N; UN – Plant N uptake in aboveground biomass at maturity (g pot^{-1}) in the pot that received N; U0 – the N uptake in aboveground biomass at maturity (g pot^{-1}) in the pot that received no N.

Statistical analysis: Data regarding growth, biomass production, N accumulation and various N-efficiency parameters were subjected to analysis of variance using STATISTIX 8.1 (Analytical Software, Inc., Tallahassee, FL, USA) following the methods of Steel *et al.* (1997), while Microsoft Office (Redmond, WA, USA) was used to graphically present the data. Differences among treatments were separated using the least significant difference test at 5% probability level.

RESULTS

1st Experiment

***NH_4^+-N and NO_3^--N release of neem coated urea (mg kg^{-1})*:** Ammonium and nitrate release of urea coated with different concentrations of neem was observed at 25 C° incubated under controlled conditions. Neem oil coated urea (NOCU)significantly decreased the urea hydrolysis to different levels depending on the rate of coating material applied. The inclinations for the inhibition of urea hydrolysis were found significantly different for the three levels. Within 14 days of incubation, maximum hydrolysis of neem coated urea occurred. There was increase in concentration of NH_4^+-N up to 14 days of incubation, then a decrease in concentration of NH_4^+-N was observed till the end of incubation period while uncoated urea showed increase in concentration up to 7 days, then a rapid decrease was observed (Figure1 A).

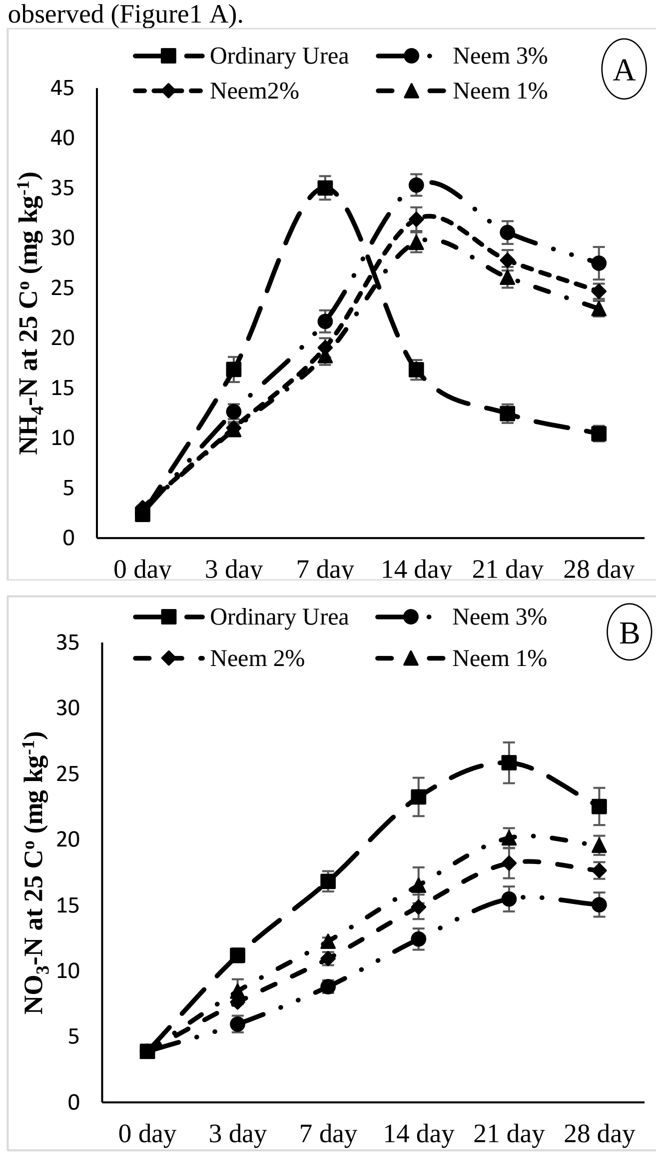

Figure 1. Effect of neem oil coated urea on NH_4^+-N and NO_3^--N release incubated for 28 days at 25 °C under controlled conditions.

Ordinary urea showed rapid hydrolysis rate which was completed at 7th days of incubation. In case of NO_3^-N release, neem oil coated urea showed increase in nitrate concentration up to 21 days of incubation, then a decrease was observed (Figure 1 B). Release rate of uncoated urea was high as compared to neem oil coated urea. Maximum concentration of NO_3^--N was observed at 1% coating level while minimum concentration was observed at 3% level of coating. Slow up gradation of NO_3^--N contents were followed by slow decrease in case of neem oil coated urea. While rapid built up in concentration of NO_3^--N was followed by rapid decrease in case of uncoated urea.

***Nitrification inhibition (%)*:** It was observed that NOCU significantly inhibited nitrification (Figure 2), to the extent of coating level applied. Maximum nitrification inhibition (41%) was observed by 3% level of coating, while minimum (21.75%) was recorded by 1% level of coating.

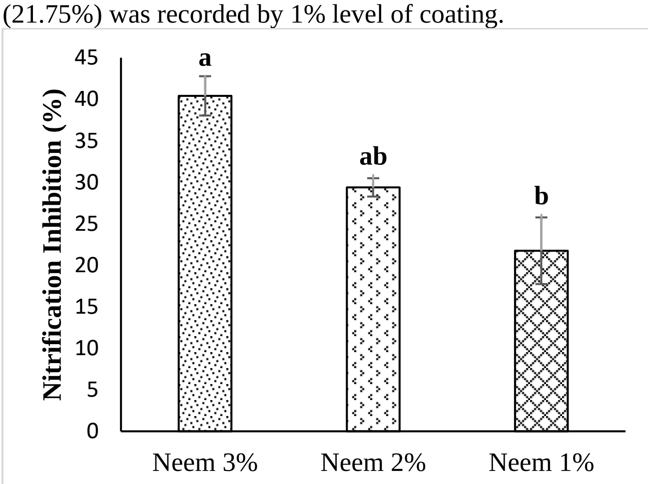

Figure 2. Effect of neem oil coated urea on nitrification inhibition, incubated for 28 days at 25 ºC under controlled conditions.

Pot Experiment

***Growth and yield characteristics*:** Table 2, shows a significant effect of coated N-fertilizer on plant height of maize. Results exposed that employing of NOCU considerably raised the plant height in comparison to ordinary urea and control. Maximum plant height of 119.33 cm was recorded against 100% level of NOCU followed by 75% level (104.33 cm) of NOCU. Application of coated urea fertilizer prominently elevated the plant dry matter of maize (Table 2) as compared to ordinary urea and control (without N). In comparison with ordinary urea, application of NOCU @ 100% and 75% level showed 15.54% and 4.24% increment in shoot dry matter, respectively. According to results, NOCU significantly increased 100 grain weight of maize (Table 2). However, treatments with 100% and 75% of recommended dose of NOCU showed highest results followed by treatments where ordinary urea was applied. Treatment with 50% of recommended dose of NOCU showed non-significant results comparatively, except control with no urea. Maximum grain yield (41.63 g) was obtained from treatment in which NOCU was applied @ 100% of recommended dose of urea (Table 2), followed by treatment where 75% level of NOCU (37.53 g) and ordinary urea (34.98 g) was applied.75% level of NOCU and full dose of ordinary urea showed comparable results and were non-significant to each other.

***Nitrogen Concentration, Grain Protein and N-Uptake*:** Nitrogen attainment in shoot was recorded maximum where NOCU was used but most significant results were recorded where 100% and 75% levels of coated fertilizer was used (Table 3). Maximum shoot N concentration was 1.59%, recorded from 100% level of NOCU which was followed by 75% level of NOCU (1.42%) while ordinary urea showed 1.34% N concentration in shoot. Similar trend was recorded

Table 2. Effect of neem oil coated urea on plant growth and yield characteristics of maize, means sharing the same letters do not differ significantly (*p*<0.05); SE ± data are average of three replicates.

Treatments	Plant height (cm)	Shoot dry matter (g)	Root dry matter (g)	100 grain weight (g)	Grain Yield (g pot^{-1})
Control	51.33±1.65 d	30.08±0.757 d	10.4±1.47 d	14.5±0.45 d	10.10±0.42 d
Ordinary Urea	94.00±1.70 b	52.81±0.68 b	23.6±1.68 bc	21.14±0.49 b	34.98±0.94 b
Neem 100%	119.33±2.12 a	60.97±1.79 a	34.6±1.44 a	23.5±0.50 a	41.63±0.68 a
Neem 75%	104.33±1.90 b	55.05±0.83 b	29.5±0.63 ab	21.80±0.53 ab	37.53±0.54 b
Neem 50%	82.33±2.37 c	45.25±1.17 c	22.7±1.02 c	18.21±0.6 c	25.12±0.37 c

Table 3. Effect of neem oil coated urea on N concentration, grain protein and N uptake by maize, means sharing the same letters do not differ significantly (*p*<0.05); SE ± data are average of three replicates.

Treatments	Shoot N (%)	Root N (%)	Grain N (%)	Grain Protein (%)	N Uptake (g pot^{-1})
Control	1.01±0.031 d	0.51±0.026 d	1.26±0.026 d	7.90±0.162 d	0.30±0.018 e
Ordinary Urea	1.34±0.015 bc	0.73±0.014 bc	1.66±0.017 bc	10.38±0.108 bc	0.70±0.016 c
Neem 100%	1.59±0.023 a	0.86±0.011 a	1.84±0.013 a	11.48±0.083 a	0.96±0.015 a
Neem 75%	1.42±0.014 b	0.74±0.015 b	1.75±0.015 ab	10.94±0.095 ab	0.78±0.018 b
Neem 50%	1.25±0.010 c	0.63±0.011 c	1.54±0.015 c	9.63±0.095 c	0.56±0.019 d

in case of N concentration in grains. All units with applied N showed significant results compared to control (without N). But most significant results were observed where 100% and 75% level of NOCU was applied (Table 3). In comparison with ordinary urea, application of NOCU showed 10.84% and 5.42% increase with 100% and 75% dose of NOCU, respectively. Similarly, application of NOCU considerably enhanced the N concentration in roots (Table 3). Although, coated urea showed significant results but highest results were obtained from 100% recommended dose of NOCU.

Protein contents in maize grains were significantly influenced by NOCU application in comparison with ordinary urea and control treatments. 100% and 75% dose of NOCU showed significant results and if compared with ordinary urea. These treatments showed 10.59% and 3.30% increase in case of 100% and 75% levels of NOCU, respectively. Nitrogen uptake depends on N availability for longer period of time. Treatments where 100% and 75% level of coated fertilizer was practiced, statistically significant results were recorded as compared to other experimental units (Table 3). According to results maximum N-uptake (0.96g pot^{-1}) was recorded from 100% level of NOCU which was followed by 75% level of NOCU (0.78 g pot^{-1}) while ordinary urea showed 0.70 g pot^{-1} of N-uptake.

***Physiological attributes*:** Figure 3 (A) revealed the influence of different levels of NOCU on photosynthetic rate of maize

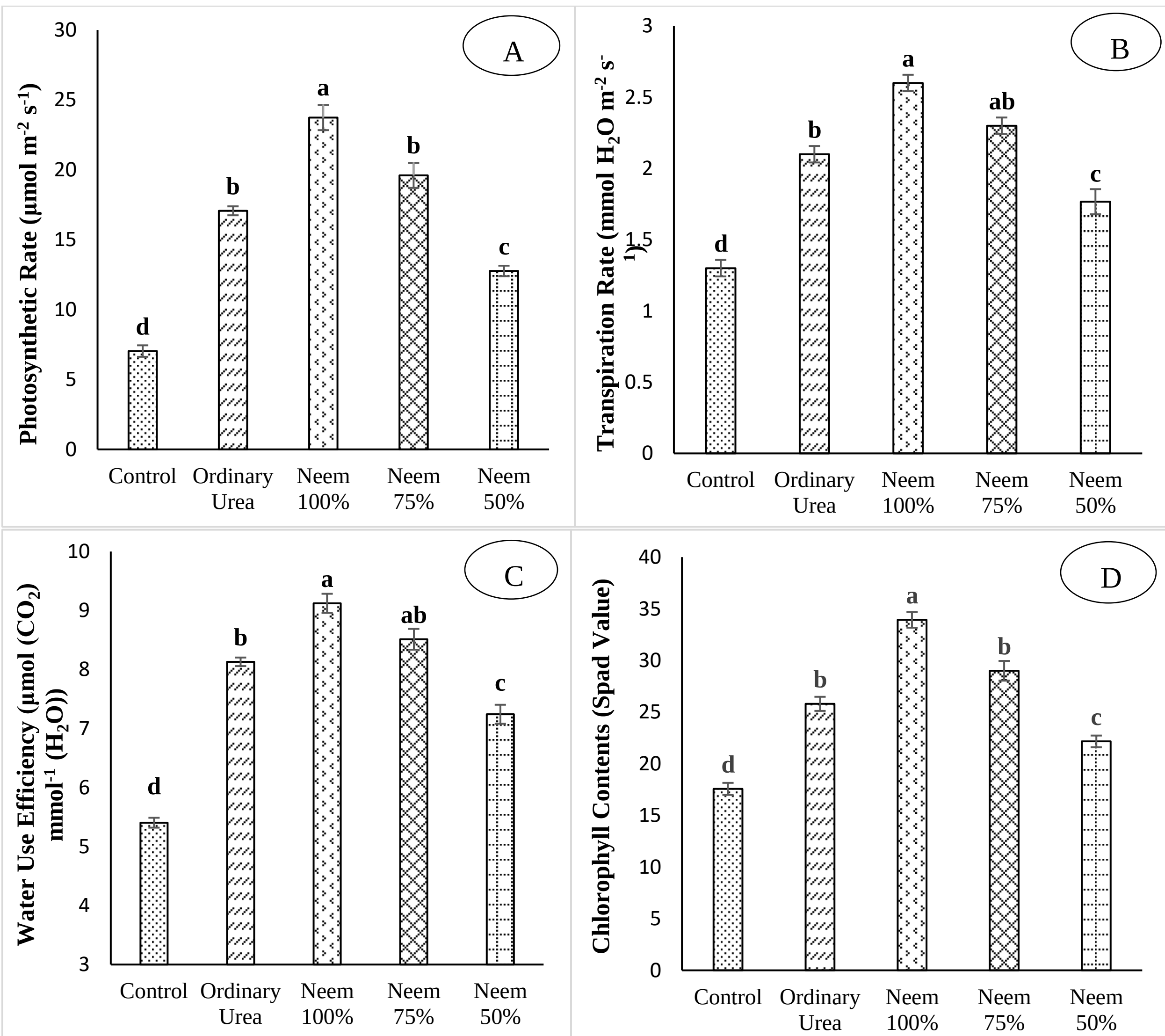

Figure 3. **Effect of neem oil coated urea on physiological attributes i.e. photosynthetic rate (A), transpiration rate (B), water use efficiency (C) and chlorophyll contents (D) of maize, means sharing the same letters do not differ significantly (*p*<0.05); SE ± data are average of three replicates.**

plants. Overall coated fertilizer showed high photosynthetic rate (μmol $m^{-2} s^{-1}$) as compared to ordinary urea and control treatment. Further, treatments containing 100% and 75% dose of NOCU showed maximum photosynthetic activity and in comparison with ordinary urea, these two treatments showed 39.01% and 14.82% increase with 100% and 75% dose of NOCU, respectively. According to presented data (Figure 3 B) NOCU significant raised the transpiration efficiency (mmol $H_2Om^{-2} s^{-1}$) as compared to ordinary urea application and control treatment. The maximum reaction in transpiration rate (2.60 mmol $H_2Om^{-2} s^{-1}$) was found for 100% level of NOCU which was followed by 2.30 mmol $H_2Om^{-2} s^{-1}$ recorded from 75% rate of NOCU. Results declared that NOCU significantly improved the water use efficiency of maize plants compared to ordinary urea. Maximum water use efficiency (μmol (CO_2) $mmol^{-1}$ (H_2O)) of maize was recorded where NOCU was applied @ 100% and 75% dose of recommended urea and in comparison with ordinary urea it showed 12.17% and 4.67% increase, respectively.

Employing of N-fertilizers significantly raised the chlorophyll contents of maize. It is evident from the figure 3(D) that where coated fertilizer was applied, increasing effect was observed and supreme chlorophyll contents were recorded from 100%, 75% dose of NOCU. In comparison with ordinary urea, 31.51% and 12.67% increment was recorded from 100% and 75% levels of NOCU, respectively. 50% dose of NOCU showed non-significant results compared to ordinary urea.

***Agronomic efficiency of applied N (AEN) (g g^{-1})*:** Maximum agronomic efficiency (24.38 g g^{-1}) of applied N was observed with 75% dose of recommended urea coated with neem oil. A decrease in agronomic efficiency of 100% level of NOCU (21.02 g g^{-1}) was observed in comparison with 75% level of NOCU (Figure 4 A). The minimum agronomic efficiency of applied N was recorded in case of ordinary urea which was 16.41 g g^{-1}.

***Recovery efficiency of applied N (REN) (g g^{-1})*:** Maximum recovery efficiency, 0.44 and 0.42 g g^{-1} was observed with 100% and 75% level of NOCU, respectively which was followed by 50% level of NOCU (0.34 g g^{-1}). On the other hand, ordinary urea showed 0.26 g g^{-1} of recovery efficiency which was non-significant to other experimental treatments (Figure 4 B).

DISCUSSION

Reduction in fertilizer use efficiency has become a serious issue for sustainable agriculture production. Efforts for breeding efficient varieties and fertilizer management practices are underway to enhance crop production and nitrogen use efficiency (NUE) (Dawson *et al.*, 2008; Baligar *et al*., 2001). Nitrification inhibitors (NIs) are among the amended N management practices used to enhance fertilizer N use efficiency due to their efficient delivery system. They have been studied under different climate conditions and farming systems to govern their performances for raising agricultural production and decreasing environmental losses (Motavalli *et al.*, 2008).

In 1st experiment, under controlled conditions highest concentration of NH_4^+-N of ordinary urea was recorded at day

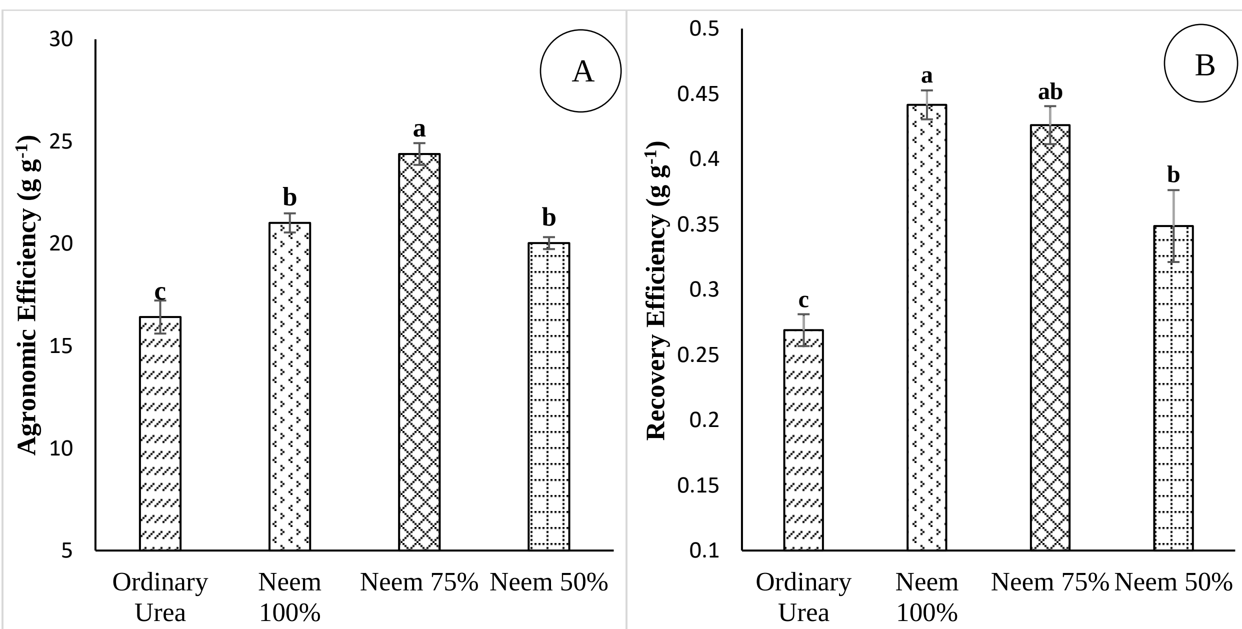

Figure 4. Effect of neem oil coated urea on agronomic (A) and recovery efficiency (B) of applied N, means sharing the same letters do not differ significantly (*p*<0.05); SE ± data are average of three replicates.

7, indicating that virtually all of the urea was hydrolyzed within the first week. While in case of NOCU, the hydrolysis was slow and maximum concentration of NH_4^+-N was observed at 14th day (Figure 1) of incubation indicating nitrification and soil urease inhibition properties of Neem. Mohanty *et al.*(2008) also reported that neem seed kernel retarded nitrification and urease activities in soil. The pattern of NH_4^+ release of NOCU was quite different from ordinary urea. The rate of NH_4^+ disappearance was much smaller in case of NOCU. At the end of incubation 27.48, 24.67 and 22.93 mg kg^{-1} of NH_4^+ was left in case of 3%, 2% and 1% level of neem coating, respectively. While ordinary urea showed only 10.44 mg kg^{-1} of NH_4^+. Application of NOCU delayed the NH_4^+ reduction and also the decrease in NH_4^+concentration was much smaller than ordinary urea. The reduction in NH_4^+ loss was due to nitrification inhibition, maintaining NH_4^+ for longer period of time (Abbasi and Adams, 2000b; Abbasi *et al*., 2011). The low NO_3^- production by NOCU indicated the nitrification inhibition by neem and high nitrate production by ordinary urea showed the high nitrification rate. Maximum nitrate was produced by ordinary urea and minimum was produced by 3% level of neem coating (Figure 1). Percent nitrification inhibition was calculated to find the performances of different levels of neem coating. Maximum nitrification inhibition (40%) was observed by 3% level (Fig. 2) of neem coating followed by 2% (29%) and 1% (22%). Majumdar (2002) reported that karanj in inhibited nitrification by 57%, 62%, 47%, 31% and 12% at 15, 30, 45, 60 and 75 days after incubation, respectively. Nitrification inhibition by neem oil coated from 4.0 to 30.9 % was recorded by Kumar *et al.* (2007). Abbasi *et al*. (2011) inferred that $Na_2S_2O_3$, neem seed cake and $CaCl_2$ inhibited nitrification in the range between 32–65%, 50–72%, and 32–70%, respectively.

Neem oil coated urea (NOCU) with 3% level of coating was selected on the basis of nitrification inhibition over 28 days of incubation for pot experiment. High dry matter yield and plant growth in coated urea pots showed that there was increased availability of N for longer period of time for the disposal of plant (Chand, 2002). Leaf expansion and number due to development of shoot through increased number of meristimatic cells by stimulating effect of nitrogen was observed (Lawlor, 2002). It is, therefore, logical to speculate that increase in plant growth was influenced by consistent supply of N as it is directly or indirectly involved in production of new cells and tissues and their enlargement which in turn increases plant biomass. Root growth was also significantly affected by NOCU under glass house experiment. The prolong availability of NO_3^- and NH_4^+ was responsible for increased root growth and its dry biomass. These mineral ions are locally sensed and activate a signaling path which stimulates elongation of lateral roots (Zhang *et al.*, 2007).

Similarly,100% dose of NOCU significantly increased the grain weight and gain yield of maize as compared to ordinary urea (Table 2). However, 75% dose of NOCU showed comparable results to ordinary urea and were non-significant to each other. Increase in yield due to coated urea application might be due to increased utilization of N by plants as a result of decreased losses of N. Adequate supply of N is one of the management approaches that improve plant growth and resultantly the grain yield (Fageria *et al.,* 2008). Kumar *et al.* (2010) observed that application of NOCU with varying thickness of coating improved the plant growth, 1000 grain weight and ultimately the yield of rice grown under different N levels.

It was also evident from results that NOCU increased the N contents in plants at 100% rates of N application over the uncoated urea (Table 3). A consistent N supply resulted in high N level in shoot which was ultimately translocated to grain. This was due to the fact that initially the N is stored in vegetative parts of plant and then it is eventually translocated to grains (Fageria*et al.*, 2003). High shoot and grain N might be contributed by N remobilization during vegetative and post-silking stage. A consistent N supply required for grain yield may increase grain N concentration to an extent, but also contribute towards high residual N levels in straw at maturity stage (Hou *et al.,* 2012).Kumar *et al.* (2010); Singh and Shivay (2003) observed increased concentration of N in shoot and grains of rice by NOCU application due to increased availability of N. The results suggested that the protein contents in maize kernel were also significantly increased by NOCU application. The prompting effect of N on protein contents in maize grains might be delivered to the direct role of N in protein formation (Taiz and Zeiger, 2006). Several research findings have revealed that increase in grain N was linked with high proportion of the monomeric gliadins as compared to large glutenin polymers, producing maximum dough extensibility and protein contents (Godfrey *et al.,* 2010; Kindred *et al.,* 2008).

Results indicated that N uptake by maize was significantly increased in the treatments supplemented with 100% and 75% dose of NOCU as compared to ordinary urea (Table 3). The increased uptake of N might be attributed to the fact that coated fertilizers were effective in synchronizing the plant N demand and fertilizer release throughout growing season (Wang *et al.,* 2015). High N uptake in coated urea treated pots was supposed to be greater utilization of N as fertilizer losses were decreased due to nitrification inhibitor application. Sing and Shivay (2003) reported high N uptake by neem application due to increased availability of N for longer period of time.

Active photosynthesis rate has always been considered a valuable characteristic during the growing season. The availability of optimum level of N is responsible for high photosynthetic rate and metabolic activity, consequently improved growth and yield (Fageria *et al.,* 2008). This investigation study has shown that application of NOCU increased the photosynthetic rate and transpiration rate as well (Figure 3). Leaf photosynthesis has been studied extensively as a plant trait in relation to NUE (Foulkes *at al.*, 2009; 2011). Maximum stomata and thylakoid membrane protein in leave is attributed to nitrogen supply that encouraged the formation of photosynthetic (Cooke *et al.,* 2005; Hikosaka, 2004). The remarkable effect of N supply on the development of chloroplasts during leaf growth has also been delivered by Hong *et al.* (2012). In turn, the chloroplast formulation leads to an increase in the lipid content of leaves and chloroplast constituents such as chlorophyll and carotene. Many investigations proved that there is very close relationship between chlorophyll contents and N (Amaliotis *et al.,* 2004). Nitrogen is a structural element of chlorophyll and also the protein molecules and thereby affects chloroplasts formation and accumulation of chlorophyll (Tucker, 2004; Daughtry *et al*., 2000). In present study the chlorophyll contents (Figure 3) were significantly increased due to NOCU application. Increased N concentration resulted in increased chlorophyll contents as these are directly related to each other (Dordas *et al.,* 2008).

Fertilizer use efficiency varies with soil properties, timing, method and amount of fertilizer being applied and also the different management practices adopted during crop development (Fageria and Baligar, 2001). NOCU significantly enhanced the nitrogen recovery and agronomic efficiency (Figure 4) of applied N. 100% and 75% levels of NOCU were equally effective in improving NUE. However, with the application of 100% dose of fertilizer, decrease in agronomic efficiency was observed as compared to 75% level. This might be attributed that the agronomic use efficiency tends to decrease as N fertilizer rates increase over a specified limit (Singh and Shivay, 2003). Results indicated that the plants supplemented with NOCU recovered more N than those which received ordinary urea. The recovery efficiency of applied N was due to the inhibition of nitrification (less chance of N losses), thereby retaining NH_4^+-N in mineral pool that may be utilized by the plants for longer period. Our results are in agreement with the previous findings (Mahmood *et al.,* 2007; Kiran and Patra, 2003; Shoji *et al.,* 2001). Kumar *et al.* (2010) established increase in N recovery and agronomic efficiency with the application of NOCU. In fact, coating of urea with neem product slowed down the availability of N from urea since neem products act as nitrification inhibitors, which resulted in increased NUE (Prasad, 2005).

***Conclusion*:** Use of nitrification inhibitors is one of the strategies to improve NUE of nitrogenous fertilizers in agricultural systems. Correspondingly, environmental risk of gaseous emission of NH_3 and N_2O and also the NO_3^- leaching can be minimized. The present study demonstrated the positive impact of NOCU on N transformations in soil. The coated urea significantly inhibited nitrification and retained mineral N in soil and extended its availability to plants. Significant improvement in maize growth and yield was observed with NOCU when applied at 100% and 75% dose of urea. Twenty-five percent N can be saved with the application of NOCU, as 75% dose of urea coated with neem oil showed non-significant results to full dose of ordinary urea.

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