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Full Length Article

Evaluating Organic Materials Coating on Urea as Potential Nitrification Inhibitors for Enhanced Nitrogen Recovery and Growth of Maize (*Zea mays* L.)

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Abstract

To slow down the urea hydrolysis and inhibition of nitrification process is a promising approach for enhancing crop production and reducing environmental risk of nitrogen (N). Two separate (incubation and pot) experiments were conducted to estimate the nitrification inhibition potential of coated urea and maize growth. The applied treatments included control, ordinary urea, neem *(Azadirachta indica)* oil coated urea (NOCU1%) and NOCU2%, moringa (*Moringa oleifera*) oil coated urea (MOCU1%) and MOCU2% and pomegranate (*Punica granatum*) leaves extract coated urea (PLECU0.5%) and PLECU1%. In incubation experiment, changes in mineral-N were studied for 40 days by analyzing NH_4^+-N and NO_3^--N contents in soil samples taken at 10-days intervals. The application of coated urea delayed the nitrification up to 30 days and increased the plant available N pool compared to un-coated urea. Highest N losses (40-48%) were observed in case of un-coated urea, while minimum NO_3^--N (7.40 mg kg^{-1}) concentrations were recorded where, 2% NOCU was applied. Maize was sown as a test crop in pot experiment with same treatments plan. Apparent N recovery ranged from 61-84% between coated urea treatments than ordinary urea. Similarly, growth related parameters i.e. plant height, dry matter yield, chlorophyll a and b significantly ($P \leq 0.05$) increased with the application of natural nitrification inhibitors (NNIs) coated urea than ordinary urea, respectively. Correspondingly, the relative growth rate (RGR) was increased by 11-89% and 30-70% in all NNIs coated urea than control and ordinary urea, respectively. In conclusion, application of NNIs seemed highly effective to reduce N losses and sustaining better crop productivity. © 2019 Friends Science Publishers

Keywords: Apparent N recovery; Mineral-N; Nitrification inhibitors; Nitrified N; Relative growth rate

Introduction

Global food security is a key challenge to feed the 9 billion people by 2050, while world food demand would be increased up to 60% (F.A.O., 2017). Almost, 50% of the world population utilize nitrogen (N) fertilizers for crop production (Erisman *et al.*, 2008). It is an important phytonutrient and is the main yield limiting factor in majority of crops (Galloway *et al*., 2004) including maize (*Zea mays* L.). Most of the soils lack plant available N, consequently, external sources of fertilization have to be applied for sustaining crop growth and yield (Jones *et al*., 2005).

One of the key agricultural practices is the higher application of nitrogenous fertilizers because of its immediate response to crop uptake. Yet, the use efficiency of the applied N in agriculture is considerably low and about 70% of the applied N fertilizer in the field is lost through one or more of different process like leaching, runoff, volatilization and de-nitrification (Subbarao *et al*., 2006). These conspicuous processes not only contribute to economic losses but also have serious concerns of environmental deterioration. For instance, increased N_2O which is one of the dominant greenhouse gas can have a considerable effect on global climate change (Zhang *et al*., 2019). Moreover, nitrate-N

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(NO_3^--N) accumulation in water bodies is a leading cause of eutrophication (Dinnes *et al*., 2002). Therefore, it is a dire need to introduce environmental friendly approaches that can minimize soil N losses and improve efficiency of applied N fertilizers.

A number of strategies are available for enhanced efficiency of fertilizers in agriculture including agronomic, molecular and genetic approaches. Use of urease and nitrification inhibitors (NIs) is one of the sustainable approaches to reduce N losses and to improve crop growth and yield (Zhang *et al*., 2019). The NIs are the chemicals or organic compounds that slow down the oxidation of urea fertilizers from ammonium (NH_4^+) to nitrate (NO_3^-) thereby delaying the activities of *Nitrosomonas* and *Nitrobacter* (Subbarao *et al*., 2006). Whereas, various chemical compounds are used as nitrification inhibitors, such as dicyandiamide (DCD) (Wakelin *et al*., 2014), 3,4-dimethylpyrazole phosphate (DMPP) and Nitrapyrin (Zerulla *et al*., 2001), but most of them are relatively expensive and have adverse effects on beneficial soil microorganisms (Dong *et al*., 2013). Therefore, it is crucial to explore the natural substances that possess the nitrification inhibitory activities for augmenting N recovery efficiency of applied fertilizers and to reduce the N losses. For example, Patra *et al*. (2002) reported that neem oil coated urea demonstrated an increase in apparent N recovery by 20-30% compared to the uncoated urea. In this regard, utilizing natural nitrification inhibitor(s) would be a viable technique to reduce soil N losses in environmental friendly manner (Prasad, 2009). Moreover, no information is available on the potential of locally available and economically feasible natural substances to inhibit nitrification and urea hydrolysis specifically, their effects on N recovery and maize growth have not been assessed. Therefore, this study was conducted (i) to evaluate the nitrification inhibition potential of selected natural substances and (ii) to determine the N recovery efficiency and impact of controlled release urea on maize growth.

Materials and Methods

Incubation Experiment

An incubation study was conducted in the wire house of Institute of Soil and Environmental Sciences (ISES), University of Agriculture, Faisalabad (U.A.F.) (31º43´ N, 73º60´ E). The soil (200 g) was taken in plastic cups from the research farm area of UAF. The soil texture was sandy clay loam (sand, 47.98%; silt, 29.52%; clay, 22.50%) and had soil pH of 7.7, soil organic matter of 0.78%, CEC of 14.5 cmol$_c$ kg^{-1}, EC of 1.2 dS m^{-1}, total nitrogen (Kjeldahl method) of 0.06%, NO_3-N (mg kg^{-1}) of 4.0 mg kg^{-1}, NH_4^+-N of 2.7 mg kg^{-1}.

Urea was developed as neem oil coated urea, moringa oil coated urea. For this 100 g of granular urea was coated with 1 mL and 2 mL of respective oil extracts representing 1% and 2% coating on v/w basis. The pomegranate leaf extract (PLE) was used with urea at the time of application into two percentages on urea granules as 0.5% and 1% (v/w). Then coated urea was allowed to dry at room temperature in Soil Fertility and Plant Nutrition Laboratory, ISES, UAF. The treatments applied were T$_1$: control, T$_2$: ordinary urea, T$_3$: neem oil coated urea (NOCU) (1%), T$_4$: NOCU (2%), T$_5$: moringa oil coated urea (MOCU) (1%), T$_6$: (MOCU) (2%) and T$_7$: pomegranate leaves extract coated urea (PLECU) (0.5%), T$_8$: PLECU (1%).

The soil was incubated for 40 days in which normal urea and urea coated with different compounds (neem seeds-extract oil, moringa seeds-extract oil and pomegranate leaves extract) were applied. These cups were incubated in the wire house of ISES, U.A.F., having daily average temperature of 22˚C (20-25˚C) and arranged in a completely randomized design (CRD) and each treatments was replicated three times. The soil moisture was adjusted by weighing the soil pot after every 2 days and lost water (>0.05 g) was added as distil water.

Nitrogen was applied in the form of urea (200 mg N kg^{-1}). Soil NH_4^+-N and NO_3^--N were analyzed in soil samples taken at 10 days intervals by AB-DTPA extract and indophenol blue methods, respectively, by using Spectrophotometer, following the International Center for Agricultural Research in Dry Areas (ICARDA) Manual (Estefan *et al*., 2013).

The nitrified N was calculated by using the following equation (Majumdar *et al*., 2001):

$$\text{Nitrified N (\%)} = \frac{NO_3^- - \text{N}}{\text{Total mineralized N}(NH_4^+ - \text{N} + NO_3^- - \text{N})} \times 100$$

The percent inhibition of nitrification for the evaluation of natural substances on coated urea was calculated as proposed by Sahrawat (1980), using the following equation:

$$\text{\% inhibition of nitrification} = \frac{C-S}{S} \times 100$$

Where, C is the NO_3^--N content in urea treated soil while S is NO_3^--N content in urea treated soil with natural nitrification inhibitors.

Pot Experiment

The same soil was used for the pot experiment. Four kg soil was taken in plastic pots and same treatments were applied as discussed previously in the incubation study. In pot study, N was applied in the form of urea (200 mg N kg^{-1}) while basal dose of phosphorus and potassium fertilizers were added in the form of superphosphate (100 mg P_2O_5 kg^{-1}) and potassium chloride (100 mg K_2O kg^{-1}). The soil was mixed thoroughly immediately after fertilizers application in each pot. Eight seeds of maize cultivar (Sygenta-8441) were sown and 4 plants were maintained at seedling stage. To maintain the constant soil moisture, distilled water was used throughout the experimental period. Two plants were harvested after 21 days of sowing and remaining two plants at second harvest (after 42 days of sowing). Data regarding growth traits like fresh and dry shoot weight, root weight, plant height, chlorophyll a & b, and total N from plants were recorded. All the laboratory analyses were carried out according to ICARDA Manual (Estefan *et al.*, 2013). The plants were harvested at two different stages and relative growth rate (RGR) was calculated following the equation proposed by Hunt (1978) as:

$$\text{RGR (mg g}^{-1}\text{ d}^{-1}) = \frac{\ln W2 - \ln W1}{\Delta T}$$

Whereas, W_1 is the short dry weight at first gravest (g), W_2 is the shoot dry weight at second harvest (g) and ΔT is the time interval between two harvest (days).

The N recovery was calculated by using the equation proposed by Singh and Shivay (2003):

$$\text{Apparent N recovery (\%)} = \frac{\text{Nt} - \text{No}}{\text{Na}} \times 100$$

Where Nt is an amount of N taken up from the test pot (mg kg^{-1}), No is amount of N taken up from the control plot (mg kg^{-1}) and Na is the quantity of added N (mg kg^{-1}).

Statistical Analysis

All the statistical analyses were performed using the SPSS v20.0 (IBM SPSS Statistics; Armonk, N.Y., U.S.A.) and graphs were plotted using Sigma-Plot v12.5. A one-way ANOVA was used to analyze the data and Duncan's multiple range comparisons (DMR) were performed to separate the difference between the means at $P \leq 0.05$.

Results

Incubation Experiment

Temporal changes in ammonical N (NH_4^+-N): The concentration of ammonium N (NH_4^+-N) in the soil increased with time under all treatments; However, increase varied significantly among the various treatments. Maximum NH_4^+-N contents were recorded after 20 days of incubation (Table 3). Maximum NH_4^+-N concentration (57.1 mg kg^{-1} soil) was observed in treatment receiving MOCU (2%) followed by NOCU (2%) and PLE (1%). After 30 days of incubation, the NH_4^+ concentration in coated treatments was still high (average 35 mg kg^{-1} soil) than non-coated urea treatment. In the soil where uncoated urea was applied, NH_4^+ concentration increased during the first 10 days and later decreased gradually with time and very little plant available NH_4^+ was left (6.5 mg kg^{-1} soil) by the end of the incubation period (Table 3). At the end of the incubation period, the treatments PLECU1% and MOCU2% maintained higher NH_4^+-N contents (20.3 and 21.1 mg kg^{-1} soil, respectively) compared to other treatments.

Temporal Changes in NO_3^--N

The NO_3^--N concentration remained statistically unchanged till 20 days after incubation and then decreased continuously in control (Table 3). The concentration of NO_3^--N increased during incubation period and reached to the maximum value of 12.61 mg kg^{-1} soil at 30 days and then decreased suddenly to 4.99 mg kg^{-1} soil in the treatments where ordinary urea was applied. Later on, the NO_3^--N concentration increased continuously, however, values varied with treatments though best performance in terms of relatively lower NO_3^--N concentration was calculated with MOCU2% (Table 3).

Percent Inhibition of Nitrification

The nitrification inhibition (NI) potential of natural substances coated on urea varied significantly among the treatments (Table 4). At 10 days of interval NOCU1% showed highest (67%) NI potential while 66% NI potential was observed in PLECU0.5% at 20 days. Similarly, at 30 days and 40 days of incubation, PLECU1% and MOCU2% showed highest NI by 53.9% and 43.9%, respectively.

Pot Experiment

Plant Growth (1st Harvest)

The plant height was significantly ($P \leq 0.05$) increased under all the urea coated treatments compared with uncoated urea (Table 1). The plant height was recorded maximum in PLECU0.5% (39.9 cm). Progressive increase in height was observed with increasing the level of coating of each treatment, except for PLECU 1% treatment in which decreased from 39.9 to 37.1 cm. The dry matter yield was not significantly different among treatments at 1st harvest.

Plant Growth (2nd Harvest)

The response of maize growth and related parameters significantly varied ($P \leq 0.05$) compared to the control (Table 1 and 2). Urea coated with different natural nitrification inhibitors produced higher dry matter, plant height and chlorophyll contents (especially chlorophyll a) relative to the ordinary urea. The maximum plant height (98 cm) was recorded in NOCU2% followed by NOCU1% (94 cm) and PLECU0.5% (91 cm) treatments. The moringa oil coated urea produced almost similar plant height at both levels i.e. MOCU1% (86.7 cm) and MOCU2% (86.3 cm). The dry matter yield of 5.09 g and 4.99 g per plant was recorded at PLECU0.5% and PLECU1%, respectively, followed by MOCU1% (3.59 g), MOCU2% (3.43 g), NOCU1% (3.42 g) and NOCU2% (3.97 g), respectively (Table 1). Similarly, root dry matter production was recorded (0.29-0.52 g $plant^{-1}$) in between coated urea treatments than ordinary urea (0.37 g $plant^{-1}$) and control (0.27 g $plant^{-1}$), respectively (Table 1).

Chlorophyll a and b also differed in each treatment (Table 2). The nitrification inhibitors (NIs) significantly ($P \leq 0.05$) increased chlorophyll contents (chlorophyll a and b) as compared to application of ordinary urea. The lowest chlorophyll a and b (21.57 and 31.14 mg g^{-1}) respectively,

Table 1: Effects of various natural nitrification inhibitors coated urea on growth related parameters of maize

Treatments	Plant height (cm)		Shoot dry weight (g/plant)		Root dry weight (g/plant)
	1st Harvest	2nd Harvest	1st Harvest	2nd Harvest	2nd Harvest
Control	25.4 ± 0.92 f	76.3 ± 1.25 g	0.28 ± 0.01 d	2.88 ± 0.08 e	0.27 ± 0.02 b
Ordinary Urea	27.6 ± 0.89 g	78.7 ± 0.47 f	0.32 ± 0.02 ab	3.01 ± 0.05 bc	0.34 ± 0.03 a
NOCU 1%	32.3 ± 0.67 a	94.0 ± 0.81 b	0.33 ± 0.03 ab	3.42 ± 0.05 de	0.36 ± 0.05 ab
NOCU 2%	38.2 ± 1.04 c	98.0 ± 0.82 a	0.35 ± 0.05 a	3.97 ± 0.04 b	0.41 ± 0.04 ab
MOCU 1%	34.7 ± 0.88 de	86.7 ± 1.25 cd	0.29 ± 0.02 ab	3.59 ± 0.03 bc	0.29 ± 0.04 ab
MOCU 2%	35.2 ± 0.8 c	86.3 ± 1.24 e	0.29 ± 0.03 ab	3.43 ± 0.04 cd	0.38 ± 0.03 ab
PLECU 0.5%	39.9 ± 1.12 e	91.0 ± 0.82 c	0.29 ± 0.02 ab	5.09 ± 0.09 a	0.47 ± 0.05 ab
PLECU 1%	37.1 ± 0.98 ab	88.0 ± 0.81 de	0.28 ± 0.02 b	4.99 ± 0.08 a	0.52 ± 0.04 a

Different lower case letters show the significance difference among the treatments ($P < 0.05$). Values are means ± S.E (n=3)
Here Control, ordinary urea; NOCU 1% and NOCU 2%, 1% and 2% urea coated with neem oil; MOCU 1% and MOCU 2%, 1% and 2% urea coated with moringa oil; PLECU 0.5% and PLECU 1%, 0.5% and 1% urea coated with pomegranate leaves extract

Table 2: Effects of various natural nitrification inhibitors coated urea (N fertilizer) on chlorophyll contents of maize

Treatments	Chlorophyll (mg g^{-1}) fresh weight	
	Chlorophyll a	Chlorophyll b
Control	17.61 ± 0.95 e	26.93 ± 0.89 e
Ordinary Urea	21.57 ± 0.86 d	31.14 ± 0.50 d
NOCU 1%	26.11 ± 0.73 c	33.71 ± 0.61 d
NOCU 2%	30.26 ± 1.31 b	34.32 ± 0.81 d
MOCU 1%	32.56 ± 1.06 a	37.05 ± 0.48 c
MOCU 2%	32.73 ± 0.87 a	40.79 ± 0.43 b
PLECU 0.5%	33.58 ± 0.90 a	42.91 ± 0.20 a
PLECU 1%	32.50 ± 0.95 a	42.56 ± 0.48 a

Different lower case letters show the significant difference among the treatments ($P < 0.05$). Values are means ± S.E (n=3)
Here Control; ordinary urea; NOCU 1% and NOCU 2%, 1% and 2% urea coated with neem oil; MOCU 1% and MOCU 2%, 1% and 2% urea coated with moringa oil; PLECU 0.5% and PLECU 1%, 0.5% and 1% urea coated with pomegranate leaves extract

Table 3: Temporal release of NH_4^+-N and NO_3- -N from urea fertilizer in a soil medium as affected by different natural nitrification inhibitor(s) (Incubation study)

	NH_4^+-N (mg kg^{-1})				NO_3^--N (mg kg^{-1})			
Treatments	10 D	20 D	30 D	40 D	10 D	20 D	30 D	40 D
Control	2.81 ± 0.10 f	1.70 ± 0.18 f	1.34 ± 0.09 g	1.31 ± 0.08 e	2.99 ± 0.06 f	1.81 ± 0.12 e	1.63 ± 0.24 e	1.58 ± 0.17 f
Ordinary Urea	20.21 ± 1.22 a	16.54 ± 0.68 e	12.25 ± 0.61 f	9.22 ± 0.63 d	4.62 ± 0.33 a	9.07 ± 0.32 a	12.61 ± 1.07 a	4.99 ± 0.61 d
NOCU 1%	7.70 ± 0.28 d	44. 89 ± 2.16 d	30.85 ± 0.56 c	15.06 ± 0.42 b	2.04 ± 0.70 de	6.36 ± 0.74 b	9.44 ± 0.70 b	10.40 ± 0.61 a
NOCU 2%	6.95 ± 0.40 d	51.44 ± 0.53 b	35.79 ± 0.55 b	12.25 ± 0.23 c	1.52 ± 0.32 e	6.22 ± 0.49 b	8.03 ± 0.84 bcd	7.40 ± 0.35 c
MOCU 1%	11.69 ± 0.55 b	50.01 ± 0.46 c	26.29 ± 0.92 f	14.94 ± 0.55 b	3.18 ± 0.17 b	3.43 ± 0.56 c	6.76 ± 1.29 cd	10.28 ± 0.24 a
MOCU 2%	10.36 ± 0.32 c	57.12 ± 0.29 a	39.54 ± 0.50 a	21.16 ± 0.45 a	2.80 ± 0.13 bc	3.06 ± 0.13 c	6.54 ± 0.78 d	7.55 ± 0.51 c
PLECU 0.5%	5.08 ± 0.53 e	49.41 ± 0.60 c	28.59 ± 0.64 e	15.16 ± 0.87 b	2.29 ± 0.43 cd	3.13 ± 0.76 c	8.27 ± 0.62 bc	9.04 ± 0.94 b
PLECU 1%	3.47 ± 1.20 f	50.83 ± 0.70 bc	30.23 ± 0.65 c	20.36 ± 0.99 a	2.50 ± 0.23 cd	2.15 ± 0.42 d	7.47 ± 0.50 cd	10.28 ± 0.24 a

The mean values ±SE (n=3), different lower case letters show the significant difference among the treatments at ($P < 0.05$), NH_4^+-N and NO_3^--N at 10 days, 20 days, 30 days and 40 days
Here Control; ordinary urea; NOCU 1% and NOCU 2%, 1% and 2% urea coated with neem oil; MOCU 1% and MOCU 2%, 1% and 2% urea coated with moringa oil; PLECU 0.5% and PLECU 1%, 0.5% and 1% urea coated with pomegranate leaves extract

Table 4: Nitrified N and percent inhibition of nitrification after the application of natural nitrification inhibitors

	Nitrified N (%)				Nitrification inhibition (%)			
Treatments	10 D	20 D	30 D	40 D	10 D	20 D	30 D	40 D
Ordinary urea	18.60 ± 0.8 c	35.44 ± 1.7 a	50.69 ± 1.0 a	29.56 ± 2.4 c				
NOCU 1%	20.74 ± 6.2 c	12.42 ± 1.6 b	23.40 ±1.1 b	40.81 ± 2.0 a	55.88 ± 8.2 ab	38.72 ± 1.3 d	32.24 ± 2.9 c	20.69 ± 3.0 c
NOCU 2%	17.90 ± 3.6 c	10.72 ± 0.8 b	18.30 ± 1.6 d	37.64 ± 1.3 a	67.15 ± 6.9 a	43.52 ± 2.5 d	40.09 ± 3.0 b	34.94 ± 2.4 b
MOCU 1%	21.40 ± 1.0 c	6.41 ± 1.0 c	20.35 ± 2.5 cd	40.77 ± 0.9 a	31.11 ± 3.8 d	51.74 ± 3.7 c	49.17 ± 3.0 a	33.54 ± 2.4 b
MOCU 2%	21.27 ± 1.1 c	5.08 ± 0.1 cd	14.17 ± 1.3 e	26.28 ± 1.3 c	39.41 ± 2.7 cd	57.23 ± 1.6 bc	52.87 ± 3.1 a	43.96 ± 1.1 a
PLECU 0.5%	31.10 ± 5.1 b	5.93 ± 1.3 cd	22.43 ± 1.7 bc	37.34 ± 3.7 a	50.27 ± 9.2 bc	60.42 ± 8.6 ab	47.97 ± 6.8 a	36.14 ± 4.6 b
PLECU 1%	43.12 ± 8.2 a	4.05 ± 0.7 d	19.81 ± 1.3 cd	33.57 ± 1.5 b	45.87 ± 4.9 bcd	66.06 ± 4.1 a	53.93 ± 0.7 a	37.07 ± 1.1 b

Different lower case letters show the significant difference among the treatments ($P < 0.05$). Values are means ± S.E (n=3)
Here Control; ordinary urea; NOCU 1% and NOCU 2%, 1% and 2% urea coated with neem oil; MOCU 1% and MOCU 2%, 1% and 2% urea coated with moringa oil; PLECU 0.5% and PLECU 1%, 0.5% and 1% urea coated with pomegranate leaves extract

were recorded by application of ordinary urea. The chlorophyll a produced by MOCU (1% and 2%) and PLECU (0.5% and 1%) was not statistically significant. The highest chlorophyll b (42.91 and 42.56 mg g^{-1}) observed by PLECU0.5% and PLECU1%, respectively. However, chlorophyll a and chlorophyll b contents were significantly different under PLECU, MOCU and NOCU application compared to ordinary urea (Table 2).

Table 5: Relative growth rate (RGR), shoot N concentration and apparent N recovery (ANR) efficiency from urea fertilizer and by application of coated urea with various natural nitrification inhibitors

Treatments	RGR (mg g^{-1} d^{-1})	Shoot N (mg g^{-1})	ANR (%)
Control	71.19 ± 1.88 c	14.02 ± 0.99 f	
Ordinary urea	79.35 ± 0.99 c	21.3 ± 1.23 e	37.25 ± 2.42 e
NOCU 1%	103.54 ± 1.53 b	34.2 ± 1.01 d	61.01 ± 3.81 d
NOCU 2%	107.83 ± 1.89 b	42.31 ± 3.20 b	75.99 ± 3.15 ab
MOCU 1%	116.81 ± 2.78 b	32.50 ± 2.76 d	65.45 ± 2.17 cd
MOCU 2%	118.01 ± 3.11 b	45.10 ± 1.87 b	73.96 ± 2.66 bc
PLECU 0.5%	134.60 ± 4.15 a	49.41 ± 1.99 a	83.98 ± 4.11 a
PLECU 1%	130.58 ± 2.36 b	39.70 ± 2.21 c	79.68 ± 2.09 ab

Different lower case letters show the significant difference among the treatments ($P < 0.05$). Values are means ± S.E (n=3)

Here Control; ordinary urea; NOCU 1% and NOCU 2%, 1% and 2% urea coated with neem oil; MOCU 1% and MOCU 2%, 1% and 2% urea coated with moringa oil; PLECU 0.5% and PLECU 1%, 0.5% and 1% urea coated with pomegranate leaves extract

Relative Growth Rate (RGR)

The relative growth rate (RGR) was increased by 11-89% and 30-70% in all coated treatments than control and ordinary urea, respectively (Table 5). The maximum RGR was determined in PLECU0.5% by 134.6 mg g^{-1} and it was 70% higher than ordinary urea followed by PLECU 1% (65%), MOCU 2% (49%), MOCU 1% (47%), NOCU 2% (36%) and NOCU 1% (30%), respectively. The RGR was statistically significant ($P \leq 0.05$) in case of PLECU 0.5% than ordinary urea and control (Table 5).

Plant N uptake and Apparent N Recovery Efficiency

Shoot N concentration was increased from 34.5% to 56.8% in treatments with natural nitrification inhibitors (NNIs) coated urea compared to ordinary urea. The PLECU 0.5% showed significantly higher shoot N concentration compared to other coated material (Table 5). On the other hand, maximum (83.98%) apparent N recovery was observed in PLECU 0.5% followed by PLECU 1% (79.68%), NOCU 2% (75.99%), MOCU 2% (73.96%), MOCU 1% (65.45%), NOCU 1% (61.01%), respectively, while lowest ANR efficiency was recorded by 37.25% in ordinary urea (Table 5).

Discussion

Plant growth and development is mostly limited by lack of available N. Therefore, management of N fertilization in agriculture is a challenging task due to a range of its influencing factors. About 70% of applied N is lost into the environment and volatilization, de-nitrification and leaching are main causes for this loss (Zakir *et al*., 2008). Nitrification of ammonical N is the initial stage resulting in loss of N to the environment. Results of this study showed that decline in NH_4^+-N concentration in soil (especially from 20-days incubation period onward) might be due to limited activities of *Nitrosomonas* and *Nitrobacter* by inhibition of nitrification through applied natural substances which are consistent with the other studies where, application of nitrification inhibitors retarded the activities of nitrobacteria (Dong *et al*., 2013; Zhang *et al*., 2019). As expected, 50% of applied N disappeared within the 30 days of incubation (Table 1). Moreover, during this period, the concentration of NO_3^--N was increased and of NH_4^+-N was decreased (Table 2), confirming that much of the NH_4^+-N was transformed into NO_3^--N in soil depicting that nitrification is the major cause of N losses (Abbasi and Adams, 2000; Abbasi *et al*., 2011). On the other hand, reduction in NH_4^+-N oxidation by using natural nitrification inhibitors (NIs) as indicated by its consistent concentration throughout a period of 30 days (incubation) in this study maybe a promising approach to inhibit the nitrification and improve the plant N uptake while decreasing its losses. It is well established that using natural substances such as neem oil having strong triterpenoid compounds controls the loss of urea-N by delaying the bacterial oxidation over a certain period of time (4 to 10 weeks) (Subbarao *et al*., 2006; Prasad, 2009). This delay in NH_4^+-N oxidation significantly varied with the applied NNIs coated urea in present study (Table 1). At the end of the incubation period (*i.e*., 40 days), the bacterial oxidation of 47% to 49.5% was delayed in all coated urea than ordinary urea, respectively. These findings, are in line with another study showing that nitrification inhibitors delayed the NH_4^+ oxidation for longer periods than urea application without nitrification inhibitors (Herrmann *et al*., 2007; Dong *et al*., 2013).

Further, the extent of nitrified N (NO_3^--N) as percent of total N in soil was much higher in treatments receiving uncoated urea (Table 4). Minimum nitrified N with NNIs was observed after 20 days of incubation period and ranged from 4.1 to 12.4% and further depends on the rate and type of the coating material (Table 4). The PLECU 0.5% and PLECU 1% proved to be stronger inhibitor in this study followed by MOCU (1% and 2%) and NOCU (1% and 2%) which is probably due to having phenolic functional groups which play an important role in delaying the nitrobacteria and urease activity (Viuda-Martos *et al*., 2010). Thus PLECU is more effective having nitrification and properties as indicated by the higher concentration of NH_4^+ for longer period of time. Percent nitrification inhibition by “NOCU and MOCU” ranged 49% to 40.9%, respectively, which is in agreement to (Patra and Chand, 2009), they described that coating urea with nitrification inhibitors significantly inhibit the nitrification and decreased the N loss. These results support the hypothesis that NNIs extends the time of N availability to plants by reducing nitrification and NH_3^+ volatilization (Patra and Chand, 2009).

The percent inhibition of nitrification is the criterion to evaluate the effectiveness of compounds to inhibit the nitrification process for curtailing N losses (Hussain *et al*., 2018). Lower values of nitrification inhibition have been related to less availability of NO_3^- with the application of NNIs (Sahrawat, 1980; Alonso-Ayuso *et al*., 2016) which is useful to limit the N losses through leaching and associated with the nitrification (Subbarao *et al*., 2006). On the other hand, one of the reason for potential nitrification inhibition in current study might be due to the fact that plant produces certain compounds which inhibit the nitrification process by blocking the activity of the ammonia monooxygenase (AMO) enzyme (Subbarao *et al*., 2006), which had profound role for augmenting nitrification rate (Jia *et al*., 2013).

Use of NNIs coated urea not only reduces the N losses in terms of nitrification but also increased the crop growth and yield (Patra *et al*., 2002). Relative to the control and ordinary urea, growth related attribute like plant height, dry matter yield (DMY) and chlorophyll content (a & b) significantly increased in coated urea, respectively. We proposed that urea coated with the NNIs significantly ($P \leq 0.05$) enhanced the growth related traits which was due to sufficient availability of N and/or slow release of urea at later stages of crop which reduce the N losses and thus provide better N utilization by maize. Likewise, a recently published meta-analysis showed that application of chemical nitrification inhibitors and slow release urea enhanced the crop growth and yield (Zhang *et al*., 2019). Besides, relative growth rate (RGR) is actually the "efficiency index" that depicts the total dry weight and per unit increase in size of plant. Plants grown with coated urea showed relatively higher RGR as compared to uncoated urea (Table 5) and this may be attributed due to slow release of N and maintaining uniform supply of N even at the later stages of crop growth (Kaleem and Manzoor, 2013). These NNIs slowed the release of N from urea granule (urea hydrolysis) for better and gradual plant availability and thus reduced the NO_3^- availability to denitrifying bacteria (Alonso-Ayuso *et al*., 2016).

The relative increase in shoot N concentration in plants grown with NNIs coated urea was between 52% and 139% over uncoated urea. Application of PLECU0.5% exhibited the highest accumulation of N in shoot of maize crop (Table 5). Generally, the plant N uptake can fluctuate vary widely and depends on the availability of N in the soil. The present study showed the higher N concentration which was due to the higher NH_4^+ concentration provided by the NNIs that retarded the bacterial nitrification and plant easily assimilated the reduced nitrate to ammonium as inorganic sources and then amino acids (Dechorgnat *et al*., 2010).

Conclusion

Relative to the ordinary urea, application of NNIs coated urea inhibited the nitrification by 43-67% and increased RGR by 30-70% and ANR recovered by 61-83%, respectively. Higher shoot N concentration and increased N recovery was mainly because of the inhibition of nitrification process and delayed the urea hydrolysis under application of urea coated with NNIs. This strategy not only enhanced the crop growth but significantly reduced the N losses and upholds the efficient use of applied N fertilizers. Consequently, results suggested that use of organic materials, which are inexpensive and easily available, should be more widely used to reduce N losses for sustaining better crop productivity.

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