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NITRIFICATION INHIBITORS IMPACT ON NITROUS OXIDE EMISSION AND AMMONIA VOLATILIZATION: A SUSTAINABLE MEASURE TOWARD A HYGIENIC ENVIRONMENT

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SUMMARY

Nitrogen (N) application to agricultural fields warrants curtailing nitrous oxide (N_2O) emission and ammonia (NH_3) volatilization for improved use efficiency with a less environmental footprint of N. As a means of mitigating N_2O emissions, the efficacy of nitrification inhibitors (NIs) is well established but the efficacy of NIs in reducing NH_3 volatilization is not well understood. The study investigated the efficacy of neem oil, pomegranate leaf extract, and dicyandiamide (DCD) coating on prilled urea for reducing N_2O emissions and the trend of NH_3 release using static air closed chamber technique in an incubation room. The results showed that all NIs reduced N_2O flux in the order of 37%–42% by DCD urea, 19%–34% by neem oil coated urea (NOCUs), and 11%–16% by pomegranate leaf extract coated urea (PLECU). However, over uncoated urea, 43%–54% NH_3 flux was increased by DCD, 10%–32% by NOCUs, and significantly the least increase (5%–14%) in NH_3 cumulative flux was shown by PLECU. Dicyandiamide significantly reduced N_2O flux more than all other treatments, and PLECU showed the least increase in NH_3 emission when compared with other coated treatments. Hence, it is suggested that neem oil and pomegranate leaf extract could be used successfully not only for mitigating N_2O emission, but also lessen environmental damages in association with managed N intense agriculture. Moreover, research focus on the increase in NH_3 volatilization using DCD needs serious attention, especially in alkaline calcareous soils.

Keywords: Coated urea, dicyandiamide, nitrous oxide, nitrogen inhibitors, neem oil, pomegranate

Key findings: Two natural and one synthetic nitrification inhibitor were assessed as coating additives for granular urea for reducing nitrous oxide emissions and ammonia volatilization. The N_2O cumulative flux was significantly decreased from 11% to 42%, however, cumulative NH_3 flux was increased from 5% to 54% by using NIs amended urea over urea treatment. Neem oil and pomegranate leaf extract are recommended as urea coating additives for reducing N_2O gas emission. However, an increase in NH_3 volatilization can be given more research focus.

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INTRODUCTION

Nitrogen (N) is not only essential for all life on earth, but also a major contributor to environmental degradation, particularly through atmospheric emissions of nitrous oxide (N_2O) and ammonia (NH_3). The N paradox requires finding solutions for the rational use of synthetic N to save input costs and the environment by reducing unwanted emissions (Norton and Yang, 2019; Bordoloi *et al*., 2020; Kirti *et al*., 2020). The emission flux is mainly influenced by the amount, form, timing, and method of applied fertilizer. In agroecosystems, nearly 15% of applied synthetic N could be lost in the form of N_2O when N fertilizer rates exceed uptake by crops (Signor and Cerri, 2013; Mahakosee *et al*., 2015). Compared with 1995, the projected estimated concentrations of NO_x (N_2O, NO, NO_2) will increase two to fourfold by 2025 (Vallack *et al*., 2001). Among the greenhouse gases, N_2O shares 20% of global warming (Olfs *et al.,* 2018; Fagodiya *et al*., 2020), and has a higher risk (300×) of global warming than carbon dioxide (IPCC, 2013; Bordoloi *et al*., 2020). Furthermore, N_2O residential time in the stratosphere is significantly higher (~120 years) than those of black carbon (~5 days) or methane (~12 years) (Millar *et al*., 2014). A little increase in atmospheric N_2O quantity may have a huge impact on global warming and stratospheric ozone depletion along with agronomic and monetary losses of N at the farm level (IPCC, 2014; Kirti *et al.,* 2020; Santos *et al.,* 2020).

Other than N_2O, a major form of N loss is the volatilization of NH_3. The NH_3 emissions drastically increased to 64% from N fertilization and are expected to reach 80% by 2030, which is equivalent to 20%–70% of the total farmland N losses (Pan *et al*., 2016; Raza *et al*., 2018). Along with farm scale losses, the volatilized NH_3 may have detrimental effects at the ecosystem scale, such as, acidification in terrestrial systems, eutrophication of aquatic systems, and decline in biodiversity (Shahzad *et al*., 2019; Fagodiya *et al*., 2020). Being one of the major atmospheric pollutants, NH_3 also poses increasing risks to human health. Different forms of N cycling like the ammonium nitrification contribute to groundwater contamination by leaching of nitrate and environmental degradation by emission of N_2O to the atmosphere (Ti *et al*., 2019; Swailam *et al*., 2021). Furthermore, NH_3 contributes to global warming by reacting with nitric acid and sulfuric acid in the atmosphere, producing secondary aerosols and haze pollution (Saggars *et al*., 2013; Raza *et al*., 2019). The NH_3 emitted from particulate matter (PM) has the potential to reduce human life expectancy by affecting respiratory tissue (Wagner *et al*., 2015). The increased death rate of humans has been reported due to increasing ammonium aerosol concentration in the atmosphere (Sutton *et al*., 2011; Ti *et al*., 2019) and the cost associated with the impact of fine particles ($PM_{2.5}$) on human health due to NH_3 aerosols is up to USD 75 per kg of N from NH_3 pollution (Stokstad, 2014; Ti *et al*., 2019).

The application of nitrification inhibitor (NI) is a viable approach to decrease N fertilizer loss and enhance nitrogen use efficiency (NUE) in agroecosystems (Wang *et al*., 2020; Bordoloi *et al*., 2020). The NIs also have a drastic influence on N_2O emissions reduction (Soares *et al*., 2012; Gilsanz *et al*., 2016) without yield penalties. The synthetic NIs like acetylene, nitrapyrin, 2-amino-4-chloro-6-methylpyrimidine, 2-sulfanilamide-thiazole, 3,4-Dimethylpyrazole phosphate, and 4-amino 1, 2, 4-triazole, have been found to reduce N_2O emission from the soil (Aulakh *et al*., 2001; Kim *et al*., 2012). The application of NI can temporarily suppress the transformation of soil ammonium (NH_4^+) to nitrate (NO_3^-). As a result, the substrate availability for NO_3 formation gets reduced and affects the denitrification process of N_2O emission (Chen *et al*., 2008).

Among plant materials, Nimin (a commercially available concentrated neem extract prepared from neem oil and neem Cake) has been reported to reduce N_2O-N emissions by 63% (Majumdar *et al*., 2002; Datta and Adhya, 2014). Pomegranate is a member of the family Punicaceae. The chemical compounds present in it possess strong antibacterial, toxicological, and pharmacological properties (Abbasi *et al.,* 2011; Behera *et al*., 2017). The powder of pomegranate fruit (PFP) can reduce urea breakdown (Al-Sabahi *et al*., 2017). Dicyandiamide, a strong synthetic nitrification inhibitor used with solid urea is produced and marketed in Japan and Germany (Hatano *et al*., 2019), which has been reported to reduce gaseous emissions significantly from the urea fertilized fields (Deklein *et al*., 2011; Zhang *et al*., 2015). But some issues are linked with the use of DCD, i.e., its high cost, availability, and toxicity (Byrne *et al*., 2020). Hence, there is a dire need to exploit some cheap and locally available natural materials that can reduce nitrogenous emissions.

Earlier, investigations have been made to assess the impact of NIs either on the emission of N_2O or methane, but the information lacks regarding the effect of NIs on NH_3 emissions (Majumdar *et al*., 2002; Zhou *et al*., 2016). In the current study, simultaneous measurements were made for N_2O and NH_3 gases to evaluate the efficacy of neem oil and pomegranate leaf extract coating on prilled urea and DCD for reduced N emissions under controlled conditions. The objectives of the study were to (i) quantify the N_2O and NH_3 emissions and (ii) evaluate the efficacy of tested substances in reducing these emissions.

MATERIALS AND METHODS

A controlled study was conducted to evaluate the efficacy of neem oil and pomegranate leaf extract coating on prilled urea for reducing the emissions of N_2O and NH_3. Their potential in lowering the NH_3 and N_2O emissions was compared with the most extensively used NI in agriculture commercially called DCD (Deklein *et al*., 2011; Zhang *et al*., 2015). Neem oil coated urea (NOCU) was developed using two variants of neem oil viz. six months old extracted (NOCU1) and freshly extracted neem oil (NOCU2) @ 500 and 1,000 mg kg^{-1}. The two variants of neem were used to compare their efficiency based on their time of extraction. Similarly, pomegranate leaf extract coated urea (PLECU) was prepared @ 500 and 1,000 mg kg^{-1}, and blended DCD urea @ 96 and 196 mg kg^{-1} was formulated (Table 1).

Table 1. Details of treatment, materials, and rates used in the study.

Treatments	Coating concentration/rate (mg kg^{-1} of urea)	Neem variants	Type of nitrification inhibitors (NIs) used	Method of coating
Control (zero N fertilizer)	-	-	-	-
Urea (160 kg N ha^{-1})	-	-	-	-
NOCU1 (neem oil coated urea)	500?; 1,000	old-extracted neem oil	NNI	Sprinkling
NOCU2 (neem oil coated urea)	500; 1,000	freshly- extracted neem oil	NNI	Sprinkling
PLECU (pomegranate leaf extract coated urea)	500; 1,000	-	NNI	Sprinkling
DCD (dicyandiamide)	96; 169	-	Synthetic	Blending

Experimental site and conditions

The trial was accomplished in the soil fertility laboratory of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. The soil was collected from the research area of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan, and the physicochemical properties of the soil were determined before the experiment (Table 2). The pilot-scale study was designed to simulate urea application conditions in static air closed polyvinylchloride (PVC) chamber as in a cropped field with some minor modifications (Leiber-Sauheitl *et al*., 2014; Olfs *et al*., 2018). A 12 kg sieved (< 2 mm), air-dried, and mechanically homogenized soil was placed in the PVC chamber as in the cropped field. The chambers were then fitted with an airtight cap having an outlet pipe. The fertilizer-soil mixture was constantly kept moist at 50% of its maximum water-holding capacity, and incubation was done at ambient temperature for 60 days in the laboratory. The experiment was conducted under a completely randomized design (CRD) with 10 treatments and three replications, for a total of 30 experimental units. The recommended dose of N (160 kg ha^{-1}) was used.

Table 2. Physicochemical properties of the experimental soil.

No.	Soil parameters	Values	Reference
	Physical properties		
1	Sand (%)	30.3	-
2	Silt (%)	40.0	-
3	Clay (%)	29.7	-
4	Textural class	Clay loam	Bouyoucos, 1962
5	Water holding capacity (%)	40	Wang *et al.,* 2020
	Chemical properties		
6	PH	8.4	U.S. Salinity Laboratory Staff, 1954
7	ECe (dS m^{-1})	0.92	U.S. Salinity Laboratory Staff, 1954
8	Available phosphorous (mg kg^{-1})	7.50	Olsen (1954)
9	Extractable potassium (mg kg^{-1})	187	Method 10a and 11a of U.S. Salinity Lab. Staff, 1954
10	Ammoniacal nitrogen (mg kg^{-1})	3.5	Tandon and Tiwari, 2009
11	Nitrate nitrogen (mg kg^{-1})	5.5	Tandon and Tiwari, 2009

Preparation of neem oil, pomegranate leaf extract coated, and dicyandiamide blended urea

The NOCU was made by weighing 20 g of neem oil in 100 mL of acetone to obtain a 20% solution of neem oil. The prilled urea was placed on a plastic sheet, and a thin film was made on it by taking 2.5 mL of prepared 20% acetone solution to get a NOCU with 500 mg kg^{-1} level of the neem-oil. Systematically mixing and air drying of NOCU was done before storage. Likewise, a 1,000 mg kg^{-1} NOCU was primed by taking 5 mL of above 20% acetonic solution of neemoil (Kumar *et al*., 2010).

To prepare the pomegranate leaf extract, a calculated quantity (1,000 g) of mature dark green leaves was taken in 10,000 mL of distilled water. The suspension was boiled for 10–15 minutes and kept soaked for three hours. The boiled suspension was filtered two times using Whatman No.40 filter paper in pressure filtration assembly. The filtrate was collected and concentrated at 55 ºC by using a vacuum rotary evaporator (Buchi-RE 121, Switzerland) to obtain crude extract powder. The PLECU was prepared by dissolving 20 g crude extract powder in 100 mL ethanol, ether, and distilled water (8:1:1) (Ibrahim *et al*., 2014; Tomar *et al*., 2015). The prilled urea was placed on a plastic sheet, and a thin film was made on it by taking 2.5 mL of prepared 20% acetone? solution to obtain a PLECU, containing 500 mg kg^{-1} dose of the extract. Systematically mixing and air drying of PLECU was done before storage. Likewise, 1,000 mg kg^{-1} PLECU was primed by taking 5 mL of above 20% solution of pomegranate leaf extract (Kumar *et al*., 2010). DCD blended urea was formulated simply by mixing 96 and 169 mg DCD with one kilogram of urea in a closed container and mixed well (Malla *et al*., 2005; Raza *et al*., 2018).

Gas measurements

Simultaneous measurements of trapped gases, i.e., NH_3 and N_2O evolved during the incubation of treated and untreated soils, were recorded. The direct measurements of N_2O were made by using a portable gas analyzer (GAS Tiger 6000 Z4 Host gas analyzer, Wandi, China) (Singh *et al.,* 2019; Bell *et al*., 2020). While the NH_3 volatilization was measured using the enclosed method using 4% boric acid solution as NH_3 absorbent (Akhtar *et al.,* 2012). The readings were recorded after two, four, 10, 20, 30, 40, 50, and 60 days of incubation (Singh *et al.,* 2013). To decrease the difference in the flux pattern, sampling was done between 10 am to 12 pm (Sapkota *et al.,* 2014).

Calculation of gas flux rates

Nitrous oxide and ammonia flux

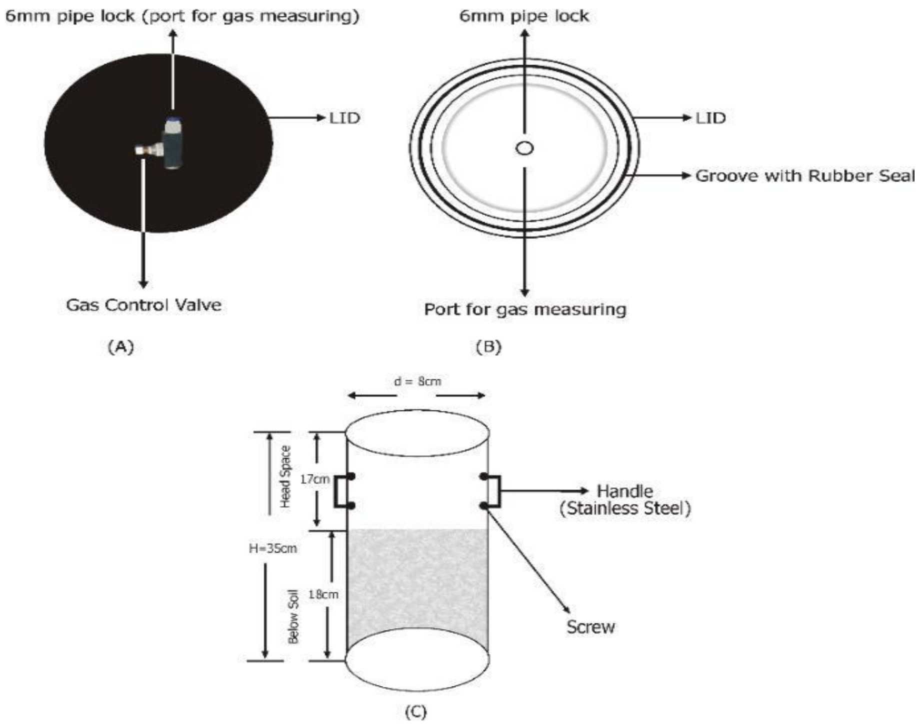
The flux rates (mg N m^{-2} d^{-1}) were calculated using the following equation (Figure 1) (Singh *et al.,* 2013; Akhtar *et al.,* 2020):

$$F = \rho \times \frac{V}{A} \times \frac{\Delta C}{\Delta t} \times \frac{273}{T + 273}$$

Where F is gas flux (mg m^{-2} d^{-1}), ρ is the density of the gas (gm^{-3}), V is the headspace volume of the chamber (cm^3), A is the base area of the chamber (cm^2), $\frac{\Delta C}{\Delta T}$ is an average rate of change in concentration for the interval (days), and T is the temperature in the chamber (K).

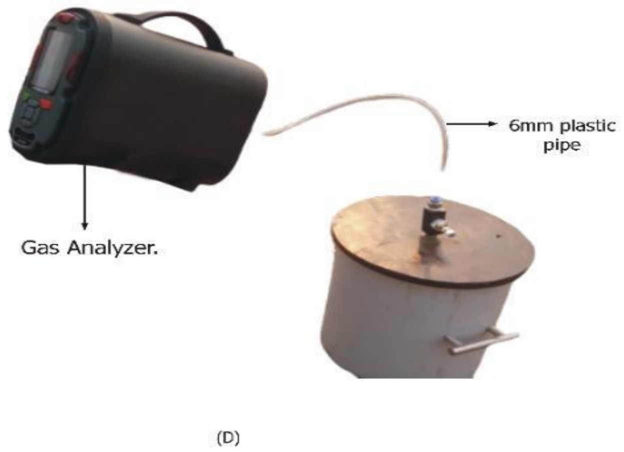
The cumulative flux

The cumulative N_2O and NH_3 flux were calculated by multiplying the average emission rate of two consecutive readings by the period length between the readings and adding that value to the previous cumulative sum (Recio *et al*., 2018).



(A) Top view of lid. (B) Internal view of lid.

(C) Schematic Design of a Static Chamber for Measuring Gas.



D: Measuring nitrous and ammonia emissions using portable gas analyzer.

Figure 1. Schematic scheme of measuring nitrous oxide and ammonia emissions using static chamber.

Percent reduction in flux

Percent reduction in the cumulative N_2O-N fluxes was determined by the following formula (Zhou *et al*., 2016):

$$\%\ \mathit{Reduction}\ \text{in}\ N_2O\ N\ \mathit{flux} = \frac{[(A-C)+(B-D)]}{(A-C)} \times 100$$

Where A is the total N_2O-N emission from urea only treatment (raw urea), B is the total N_2O-N emission from urea + NI treatment, C is the total N_2O-N emission from control (without any treatment), D is the total N_2O-N emission from N only treatment (A) minus the total NH_3-N emission from control (C).

Percent increase in ammonia flux

The percent increase in the cumulative ammonia nitrogen (NH_3-N) fluxes over urea was calculated from the following formula:

$$\%\ Increase\ in\ NH_3\ N\ flux = \frac{A-C}{C} \times 100$$

Where A is the total NH_3-N emission from urea + NI treatment and C is the total NH_3-N emission from urea (raw urea).

Statistical analyses

Significant means were evaluated by variance analysis (ANOVA) and using Tukey HSD in all pairwise comparison tests. Statistical significance was determined at the ɑ 0.05 probability level. Statistical analyses were performed using Statistix 8.1 version (USA) (Singh *et al.,* 2013).

RESULTS

Emission of ammonia

The NH_3-N cumulative flux ranged from 13 to 68 mg m^{-2} during 60 days of the study in different treatments with a total loss of 4.95 kg NH_3-N ha^{-1}. A small NH_3-N daily flux was observed on day two after the application of fertilizers in all the experimental units containing urea and urea plus nitrification inhibitors. The highest peak of NH_3-N emission was observed on day four in all the treatments. After day four, this peak declined and reached a low level of NH_3-N flux in all the treatments on day 60. The soil was incubated for 60 days. All the means were significantly different from each other on days two, four, 10, and 20, and on days 30, 40, 50, and 60, means were not different statistically from each other. The honest significant difference (HSD) (P = 0.05) was 0.82, 0.69, 0.76, 0.70, 0.78, 1.07, 1.07, and 0.93 for two, four, 10, 20, 30, 40, 50, and 60 days, respectively.

The data given in Figure 2 indicated that the daily NH_3-N flux ranged between 0.40 to 3.57 mg m^{-2} day^{-1} in the control treatment, which was between 0.46 mg m^{-2} day^{-1} to 15.38 mg m^{-2} day^{-1} in uncoated urea. For the coated treatments, the daily NH_3-N flux ranged between 0.56 to 16.0 mg m^{-2} day^{-1} in NOCU1 used @ 500 mg kg^{-1}; in NOCU1 where it was used @ 1,000 mg kg^{-1} the daily NH_3-N flux ranged between 0.56 to 16.93 mg m^{-2} day^{-1}. As for NOCU2 (500 mg kg^{-1}), the daily NH_3-N flux ranged between 0.56 to 16.26 mg m^{-2} day^{-1}, and where it was used @ 1,000 mg kg^{-1}, the daily NH_3-N flux ranged between 0.62 to 18.0 mg m^{-2} day^{-1}. Similarly, in PLECU, the range of daily NH_3-N flux was 0.47 to 14.99 mg m^{-2} day^{-1} and 0.48 to 15.59 mg m^{-2} day^{-1} in 500 mg kg^{-1} and 1,000 mg kg^{-1}, respectively. In DCD @ 96 mg kg^{-1}, the daily NH_3-N flux was ranged between 0.66 to 20.86 mg m^{-2} day^{-1}, and 0.68 to 21.83 mg m^{-2} day^{-1} @ 169 mg kg^{-1} coating concentration.

A higher coating concentration produced more NH_3 emissions than a lower coating rate. During the entire experimental period, higher NH_3 daily flux was calculated in DCD treatment applied at a higher coating rate of 169 mg kg^{-1}, followed by the NOCU2 @ 1.000 mg kg^{-1}, NOCU1 @ 1,000 mg kg^{-1}, and PLECU @ 1.000 mg kg^{-1}. The urea only treatment and the lowest daily flux was observed in control. Similarly, at a lower coating concentration (96 mg kg^{-1}), DCD maintained more NH_3 daily flux than all other treatments, which was followed by the NOCU2 @ 500 mg kg^{-1}, then NOCU1 @ 500 mg kg^{-1}, with PLECU @ 500 mg kg^{-1} showing the lowest increase in NH_3 daily flux (Figure 2).

An increase in cumulative NH_3-N flux over urea only treatment was observed in all the urea plus NIs treatments at varying levels. Irrespective of the coating concentration, the percent increase in NH_3-N cumulative flux by NOCU1 ranged between 10% to 22%, 13% to 32% by NOCU2, and 5% to 14% by PLECU, while it ranged from 43% to 54% by DCD. Resultantly, the maximum percent increase over urea in NH_3-N cumulative flux during 60 days of the incubation was observed by DCD, and PLECU @ 500 mg kg^{-1} showed the least increase in NH_3-N cumulative flux (Table 3).

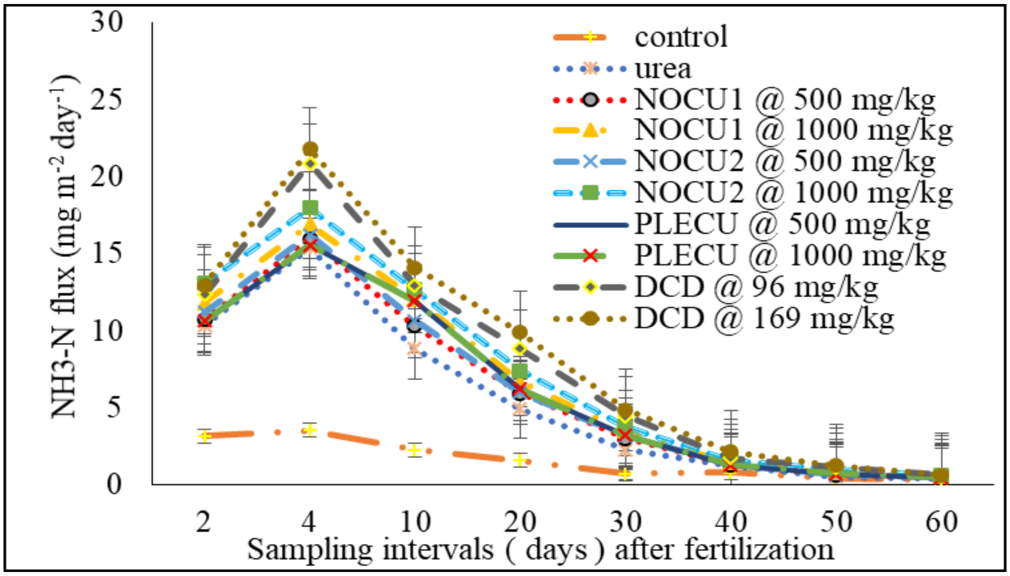


Figure 2. Daily ammonia flux (mg m^{-2} day^{-1}) as affected by the application of neem oil coated urea (NOCU1 and NOCU2), pomegranate leaf extract coated urea (PLECU), and dicyandiamide (DCD) amended urea. Vertical bars indicate the standard error.

Table 3. Percent reduction in cumulative N_2O flux and percent increase in cumulative NH_3 flux, over urea as affected by different nitrification inhibitors.

Treatments	Reduction in N_2O cumulative flux (%)	Increase in NH_3 cumulative flux (%)
Control	-	-
Urea	-	-
NOCU1 (neem oil coated urea) @500 mg kg^{-1}	19	10
NOCU1 (neem oil coated urea) @1,000 mg kg^{-1}	27	22
NOCU2 (neem oil coated urea) @500 mg kg^{-1}	22	13
NOCU2 (neem oil coated urea) @1,000 mg kg^{-1}	34	32
PLECU (pomegranate leaf extract coated urea)@500 mg kg^{-1}	11	5
PLECU (pomegranate leaf extract coated urea)@1,000 mg kg^{-1}	16	14
DCD (dicyandiamide) @96 mg kg^{-1}	37	43
DCD (dicyandiamide) @169 mg kg^{-1}	42	54

Emission of nitrous oxide

The N_2O-N cumulative daily flux ranged from 0.021 to 7.33 mg m^{-2} during 60 days of the study among used treatments with a total loss of 0.53 kg N_2O-N ha^{-1}. A small N_2O-N daily flux was observed on day two after the application of treatments in all the experimental units containing urea and urea plus nitrification inhibitors. The highest peak of N_2O-N daily flux was observed on day four in all the treatments. After day four, this peak declined and reached a low level of N_2O-N flux in all the treatments on day 60. The honest significant difference (HSD) ($P = 0.05$) is 0.66, 0.73, 0.80, 0.88, 1.00, 1.24, 0.74, and 0.84 for two, four, 10, 20, 30, 40, 50, and 60 days, respectively. The data showed that the daily N_2O-N flux ranged between 0.0 to 0.01mg m^{-2} day^{-1} in control was between 0.0 to 7.53 mg m^{-2} day^{-1} in uncoated urea (Figure 3). Meanwhile, in coated treatments, the daily N_2O-N flux ranged between 0.0 to 0.89 mg m^{-2} day^{-1} in NOCU1 used @ 500 mg kg^{-1}; in NOCU1, @ 1,000 mg kg^{-1}. The daily N_2O-N flux ranged between 0.0 to 1.31 mg m^{-2} day^{-1}.

As for NOCU2 (500 mg kg^{-1}), it was ranged between 0.0 to 1.08 mg m^{-2} day^{-1}, and @ 1.000 mg kg^{-1}, the daily N_2O-N flux ranged between 0.0 to 1.96 mg m^{-2} day^{-1}. Similarly, in

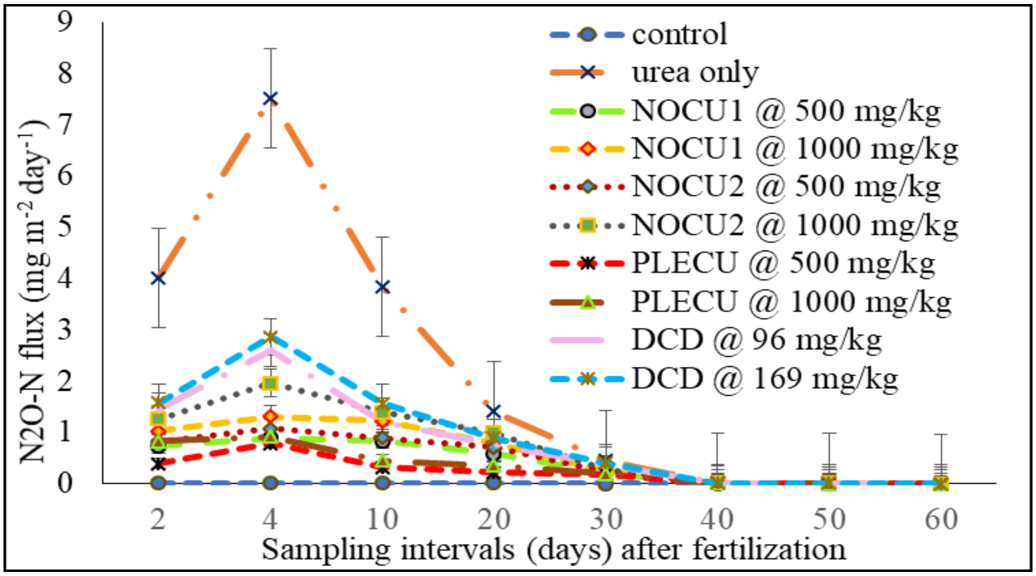


Figure 3. Daily nitrous oxide flux (mg m^{-2} day^{-1}) as affected by the application of neem oil coated urea (NOCU1 and NOCU2), pomegranate leaf extract coated urea (PLECU), and dicyandiamide (DCD) amended urea. Vertical bars indicate the standard error.

PLECU the range of daily N_2O-N flux during 60 days of the study was 0.0 to 0.78 mg m^{-2} day^{-1} and 0.0 to 0.92 mg m^{-2} day^{-1} in 500 mg kg^{-1} and 1,000 mg kg^{-1}, respectively. In DCD (96 mg kg^{-1}), the daily N_2O-N flux was ranged between 0.0 to 2.60 mg m^{-2} day^{-1} and 0.0 to 2.86 mg m^{-2} day^{-1} in DCD (169 mg kg^{-1}) (Figure 3).

The data shown in Table 3 indicated that all the NIs used in this study were found effective in decreasing N_2O-N daily flux at varying degrees. Maximum cumulative N_2O-N flux (17 mg m^{-2}) was shown by raw urea followed by DCD (@ 169 mg kg^{-1}) which showed N_2O-N flux (7 mg m^{-2}) and the least (0.02 mg m^{-2}) N_2O-N cumulative flux was found in control. Regardless of the coating concentration, the percent decrease in cumulative N_2O-N daily flux by NOCU1 ranged between 19% to 27%, 22% to 34% by NOCU2, and 11% to 16% by PLECU, while it ranged from 37% to 42% by DCD. A maximum percent decrease over urea in N_2O-N cumulative daily flux during 60 days of the incubation was indicated by DCD, followed by the NOCUs. The PLECU showed the least percent decrease in reducing N_2O-N cumulative flux.

DISCUSSION

Ammonia and nitrous oxide emissions characterize agronomic losses due to the low efficiency of N fertilizers contributing to environmental deterioration (Saggar *et al*., 2013). This signifies the worth of monitoring N emissions from today's managed systems of agriculture (Pan *et al*., 2016). The use of NIs is a proven technique worldwide for reducing N_2O emissions and enhancing N-use efficiency in agroecosystems (Gilsanz *et al*., 2016). But their availability, cost, and adaptability by the farmers are big issues linked with their widespread use (Adair and Schwartz, 2008). The investigations of this study revealed that by using some cheap, natural, and locally available materials like NIs, these issues might be addressed for sustainable production of food. Though, a more renowned effect in reducing N_2O emissions was shown by DCD.

The highest daily NH_3 emission on day four after fertilization might be due to immediate intensive hydrolyses of urea in soil. Daily NH_3 emission and flux were high in the treatments where coated urea was applied as compared to uncoated urea throughout the study period (Table 3). It indicated that the used NIs inhibited the nitrification process and holds the NH_4-N for a longer period. This increased NH_4-N pool provided the base for more NH_3 daily emission and flux than urea alone. The stronger the effect of NIs coated material, the higher the rate of nitrification inhibition, and hence, more pool of NH_4-N was available for greater NH_3 flux. The prolonged NH_4^+ retained in soils treated with NIs increased NH_3 emission from soil (Qiao *et al*., 2016; Raza *et al*., 2019). Comparatively, less daily NH_3-N emission and flux by both the variants of neem oil coated urea (NOCUs) and pomegranate leaf extracted coated urea (PLECU) over DCD was due to their extra benefit of retarding urea hydrolysis along with the inhibition of nitrification (Kumar *et al*., 2007; Kumar *et al*., 2010; Cantarella *et al*., 2018).

The significantly less increase in NH_3 emission by PLECU (Table 3) was due to its extra benefit of retarding urea hydrolysis (Ismail *et al*., 2016; Behera *et al*., 2017). It acted largely as a urease inhibitor (UI) than NI. The urease inhibitors significantly decrease NH_3 emission loss. This might be linked to the inhibition of urease enzyme by UIs, which slows down urea hydrolytic breakdown. Several synthetic UIs have been explored to retard urea hydrolysis and for mitigating the N emissions (Pan *et al*., 2016; Modolo *et al*., 2018). Slow/controlled fashioned urea generally cuts N loss via the polymerization of urea or through the addition of functional groups to urea (Yamamoto *et al*., 2016).

The results of the current study showed a maximum percent increase in NH_3 cumulative flux over urea by DCD. Moir *et al*. (2012) and Zhou *et al*. (2015) reported higher accumulative NH_3 emission in the plots where DCD was applied, as compared with urea-only treatment. Nastri *et al*. (2002), Soares *et al*. (2012), Recio *et al*. (2018), and He *et al*. (2018) reported an increase in NH_3 volatilization by the application of DCD, along with urea, over urea alone. In earlier research, Sommer *et al*. (2004), Soares *et al*. (2012), Zaman and Nguyen (2012), and Qiao *et al*. (2016) reported increased NH_3 loss through volatilization while using NIs along with urea in the soil.

The results of the current study showed that all the tested NIs were effective in reducing the N_2O-N emission to varying extent (Table 3). The natural bacterial nitrification and denitrification process happening in managed intense agricultural and unmanaged soils are major factors toward global emissions of nitrous oxide (Braker and Conard, 2011). During the nitrification process, urea ammonium (NH_4^+) is converted initially to nitrite (NO_2^-) and then, NO_3^- is formed by Nitrosomonas and Nitrobacter nitrifying bacteria of nitrosamines specie (Leininger *et al*., 2006). The nitrifiers produce an enzyme called ammonium monooxygenase (AMO) which drives the nitrification. The low N_2O-N emission might be due to that the NIs bear a Thiono-S functional group that binds to the copper (Cu) present in the active sites of microbial AMO enzyme while some stop the AMO by disrupting their heterocyclic ring of N (McCarty, 1989; Norton and Ouyang, 2019).

Nimbin, a commercial product prepared from the extract of neem kernels, has been revealed to decrease urea N_2O emissions (Datta and Adhya, (2014); Malla *et al*., 2005). Bronson *et al*. (1994), Hala *et al*. (2014), Gupta *et al*. (2016), and Bordoli *et al*. (2020) concluded that neem oil can inhibit nitrification rate in soil application and consequently, N_2O emission from the soil. Al-Sabahi *et al*. (2017) concluded that the powder of pomegranate fruit (PFP) had little effect on nitrification and the production of NH_4-N and NO_3-N + NO_2-N was decreased. Stabilized urea amended with PFP reduced ammonia emission and increased N persistence (Kiran and Patra, 2003; Chagas *et al*., 2016).

The DCD has been investigated for over 80 years for its outstanding nitrification inhibitory properties in agricultural soils (Zhang *et al*., 2015, Hatano *et al*., 2019). The outcomes of this experiment agreed with the results of De Klein and Eckard (2008), and Malla *et al*. (2005), who reported reductions in direct N_2O release from animal urine, livestock slurry, and urea along with DCD in the soil. Less N_2O emission from the DCD treatment when compared with normal fertilization was recorded (Zhou *et al*., 2016; Liu *et al*., 2017; Fan *et al*., 2018).

CONCLUSIONS

The DCD was found most effective in reducing N_2O-N emission, however, it also increased significantly higher NH_3 emissions among all other chemicals. The PLECU represented the least increase in NH_3 emission and the least reduction in N_2O emission than NOCUs and DCD. Hence, it is suggested that neem oil and pomegranate leaf extract might be encouraged at farmer's and policymakers' levels to overcome the environmental damages concerned with the use of the essential element, nitrogen, in the current era of agriculture. Therefore, the exploration of the nitrification and urease inhibitory potential and scope of some other locally available plant-based natural materials is necessary for future studies for their widespread use by the farmers on the national level. However, the increase in NH_3 volatilization using NIs can be further investigated.

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