Original Research Article

The effect of the use of a novel urease inhibitor coated urea on the greenhouse gas emissions and ammonia volatilization losses from a maize field

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ABSTRACT

Aims: To evaluate the effect of urea coated with cashew nut shell liquid, a urease inhibitor on the gaseous emissions (nitrous oxide, carbon dioxide, ammonia) from soil under maize cultivation.

Study design: Randomized Block Design

Place and Duration of Study: Division of Environment Science, ICAR- Indian Agricultural Research Institute, Delhi between November 2019 and October 2021.

Methodology: The study compared the greenhouse gas emissions and ammonia volatilization losses from a maize field fertilized with (a)prilled urea, (b)neem oil coated urea (NCU) and (c) cashew nut shell liquid coated urea (CCU). A plot with no applied nitrogen served as the control. Gaseous emissions were measured using the closed chamber method.

Results: CCU showed a 15.84% decrease in ammonia volatilization and NCU had 9% more emissions compared to that of urea. Cumulative greenhouse gas emissions varied among the treatments in the order urea> CCU> NCU> Control. CCU treated plots exhibited a net GWP decrease of 8.59% compared to urea and an increase of 4.13% compared to NCU.

Conclusion: Cashew nut shell liquid coated urea had a significant impact on the reduction of ammonia volatilization losses, hence identifying it as a viable option for use in cereal crops like maize.

Keywords: urease inhibitor, cashew nut shell liquid, ammonia volatilization, greenhouse gas, maize

1. INTRODUCTION

Nitrogen (N) is a crucial macronutrient required for plant growth and development, playing a vital role in various physiological processes such as photosynthesis, protein synthesis, and chlorophyll production. In the agricultural sector, nitrogen is commonly applied in the form of synthetic fertilizers or organic manures to supplement the natural nitrogen availability in the soil and optimize crop yields. In order to meet the demands of a growing population and increasing food production, the use of nitrogen fertilizers has become ubiquitous in modern agriculture. However, the mismanagement or overuse of these fertilizers can lead to significant environmental concerns, such as water pollution, greenhouse gas emissions, and disruption of natural ecosystems. In 2019, the application of synthetic nitrogen (N) fertilizers contributed to 8.3% of the total emissions at the farm level. When nitrogen fertilizer is added to the soil, only a fraction is absorbed by the plants. A fraction of the nitrogen is utilised by soil microorganisms, which generate N_2O as a by-product of their metabolic processes. Additionally, a portion of the applied nitrogen may be lost through leaching or volatilization from the site of application. Soil microbial activity emit N_2O, a greenhouse gas (GHG) that has 298 times greater global warming potential than CO_2 over a period of 100 years. Implementing measures to decrease the amount of N_2O emissions per unit of N compound applied is a viable approach to mitigate the worldwide influence of N fertilizers on human-induced greenhouse gas emissions. According to the FAO, the global consumption of synthetic nitrogen fertilizers is projected to rise by 50% from the 2012 level by the year 2050. Consequently, there will be a substantial rise in N_2O emissions from agricultural soils, posing a potential risk to the climate goal set by the Paris Agreement of limiting global warming to 1.5 °C or much below 2 °C above pre-industrial levels.

Synthetic nitrogen fertilizers are produced through energy-intensive industrial processes, often relying on fossil fuels, which contribute to the overall carbon footprint of agricultural production [1]. In addition, the applied nitrogen is lost to the atmosphere as ammonia or nitrous oxide, or leaches into groundwater and surface water bodies, causing eutrophication and contamination of drinking water supplies[2]. It is estimated that only around 10-50% of applied nitrogen is utilized by crops, with the remaining fraction being lost to the environment[3].

To address these environmental challenges, researchers have explored alternative strategies to improve nitrogen use efficiency and reduce the environmental impact of agricultural practices. One approach is the use of urease inhibitors or nitrification inhibitors, which can slow down the conversion of applied nitrogen into more mobile and reactive forms, thereby reducing losses[2]. Several nitrification inhibitors have been evaluated for their potential to mitigate nitrous oxide emissions and nitrate leaching in different cropping systems. Some of them are synthetic in origin, like dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP), while others are derived from natural sources, such as neem extracts and pyrolysis products. Although the synthetic nitrification inhibitors are found to have significant impact on the nitrous oxide emissions, the long-term sustainability and environmental impacts of their use are still under investigation[3]. The cost of production of these inhibitors is also high, limiting their widespread adoption in developing countries.

Urease is an enzyme that catalyses the hydrolysis of urea, converting it to ammonium. This rapid hydrolysis can lead to increased ammonia volatilization, which can result in significant nitrogen losses from agricultural systems. Researchers have explored various strategies to mitigate these losses, such as the use of urease inhibitors and the application of alternative nitrogen sources.

Urease inhibitors, such as N-(n-butyl) thiophosphorictriamide, have been shown to effectively reduce ammonia volatilization by slowing down the hydrolysis of urea.Plant-derived compounds have also been investigated as potential urease inhibitors, as they may offer a more environmentally friendly and sustainable solution. However, the identification of suitable and cost-effective candidates remains a challenge. [4]. Exploring natural sources of urease and nitrification inhibitors from plants could provide a more sustainable and cost-effective alternative to synthetic options, potentially reducing the environmental impact of nitrogen fertilizer use in agriculture.

Cashew Nut Shell Liquid (CNSL) is a versatile by-product of the cashew industry. The nut has a shell of about 1/8inch thickness inside which is a soft honey comb structure containing a dark reddish brown viscous liquid. It is called cashew nut shell liquid, which is the pericarp fluid of the cashew nut. It is often considered as the better and cheaper material for unsaturated phenols.

CNSL has innumerable applications in polymer based industries such as friction linings, paints and varnishes, laminating resins, rubber compounding resins, cashew cements, polyurethane based polymers, surfactants, epoxy resins, foundry chemicals and intermediates for chemical industry. [5] found the urease inhibitory activity of anacardic acid and cardol, the major constituents of CNSL.Anacardic acid alone constitutes about 90% of the cashew nutshell liquid (CNSL), and the remaining part is constituted by related compounds such as cardanol, cardol and 2-methyl cardol. It is a yellow liquid partially miscible in alcohol and ether but nearly immiscible in water. Chemically, anacardic acid is a mixture of several closely related organic compounds each consisting of a salicylic acid substituted with saturated or unsaturated alkyl chain that has 15–17 carbon.But its potential as a candidate for producing an enhanced efficiency fertilizer and the impact of its use on the ammonia volatilization as well as the greenhouse gas emissions have not been explored much in previous studies. Hence, the present study aims to produce a CNSL coated urea and evaluate its effects on GHG and ammonia volatilization losses from maize field in the Indo-Gangetic Plains, one of the regions of the country where excessive use of N fertilizer is prevalent.

Improving nitrogen use efficiency is crucial for both agricultural productivity and environmental sustainability. Strategies that can reduce ammonia volatilization losses, such as the use of enhanced-efficiency fertilizers and the optimization of application practices, are essential for minimizing the negative impacts of agriculture on the environment [6], [7].

2. MATERIAL AND METHODS

2.1. Experimentalsiteandsoil

A field experiment was carried out in 2020-21, where maize was grown during the Kharif season of 2020 and 2021. The study took place at the experimental farm of the Indian Agricultural Research Institute, located in New Delhi, India. The site is situated in the Indo-Gangetic alluvial tract at coordinates 28°40'N and 77°12'E, with an elevation of 228 m above mean sea level. The region has a subtropical, semi-arid climate. The region experiences a yearly precipitation of 750mm, with around 80% of it occurring between June and September. The average maximum and minimum temperatures recorded from July to October range from 35 to 18°C. The soil at the experimental site was a Typic Ustochrept, composed of loam, with a texture consisting of 46% sand, 33% silt, and 21% clay. It had a bulk density of 1.38 g cm^{-3}, a pH of 8.2 (measured using a 1:2 soil to water ratio), an electrical conductivity of 0.45dS m^{-1}, a cation exchange capacity (CEC) of 7.4 Cmol $(p^+)kg^{-1}$, and contained 4.5 g kg^{-1} of organic carbon, 0.30 g kg^{-1} of total nitrogen, 0.007 g kg^{-1} of Olsen phosphorus, and 0.13 g kg^{-1} of potassium extractable with ammonium acetate.

2.2. Preparation of Cashew Nut Shell Liquid coated urea

The cashew nut shell liquid for coating was procured from the market. The concentration of CNSL to be used for coating and the methodology for coating was standardized after conducting a series of lab experiments. To prepare CNSL coated urea for field application, 0.2% Cashew Nut Shell Liquid (CNSL)by weight was dissolved in about 20ml of ethanol and added to 1kg of prilled urea in a Schott bottle. The bottle was vigorously shaken to mix and the contents were spread on to a tray in order to evaporate the solvent. The prepared fertilizer was stored in air tight bags until further use in the field.

2.3. Crop management and treatments

The maize crop was sown on 9th July in the first year and 27th June in the second year. The crop variety used was Pusa Jawahar Hybrid Maize- 1. The seeds were sown with a row-row spacing of 30cm and plant-plant spacing of 20cm. The experimental design was Randomized Bock design. The experiment included 4 treatments with five replications in plots of 4 m long and 3m wide (Table I). The nitrogen fertilizers were applied at the rate of 150 kg N ha^{-1}, in 3 splits. 50% of total N was applied at knee height, 25% at tasselling and 25% at grain filling stage.Phosphorus (60kg P ha^{-1}) and Potassium (40 kg K ha^{-1}) fertilizers were applied as basal dose through Single superphosphate and Muriate of potash respectively. The crop was irrigated before sowing as well as a day before fertilizer application, when the rainfall was not sufficient to maintain a soil moisture of 50 % field capacity.

Table 1 Details of treatments used in the study

Treatment No.	Treatment details	Abbreviation	No. of replications
1	No nitrogen- Control	Control	5
2	Recommended dose of nitrogen (RDN) from Prilled Urea	U	
3	RDN from Neem Coated Urea	NCU	
4	RDN from CNSL Coated Urea	CCU	

2.4. Collection and analysis of gas samples

Collection of gas samples was carried out by the closed chamber technique [8], [9]. Gas sampling was done on every day for a week after fertilizer application, alternate days in the week after and twice a week afterwards. Concentrations of CO_2 and N_2O in the gas samples were estimated by Gas Chromatograph (Agilent GC7890A Agilent, Germany) fitted with amicro electron capture detector (μECD) andFlame Ionization Detector (FID) and 6' x 1/8" stainless steel column (Porapak N). Column, injector, and detector temperatures were 50 °C, 120 °C, and 320 °C, respectively. Carrier gas was N_2 with a flow rate of 14 ml min^{-1}. The ammonia volatilization losses were estimated by the forced air draft method [10]. The ammonia was trapped in a solution of 0.5% boric acid with mixed indicator. The solution was back titrated with 0.02N sulphuric acid to determine the amount of ammonia volatilized. The measurement of ammonia volatilization was carried out on every day after fertilizer application for one week, after which the emissions were at lower values, undetectable by the current method.

2.4.1. Emission of greenhouse gases from soil

Estimation of total CO_2 and N_2O emissions during the crop season was done by successive linear interpolation of average emissions on the sampling days assuming that the emissions followed a linear trend during the periods when no sample was taken [11].

2.5. Soilanalysis

Soil sampling was done at the beginning of each crop season to estimate the physico-chemical properties. Soil samples from the 0–15 cm soil layer in 3 locations in each plot were collected using a core sampler. The entire volume of soil was weighed and mixed thoroughly and a subsample was taken to determine dry weight. The fresh soil was air-dried for 7 days, sieved through a 2 mm screen, mixed, and stored in sealed plastic jars for analyses. Representative subsamples were drawn to determine physico-chemical properties using standard procedures [12].

2.6. Estimation of yields of maize

Maize yield was determined from the total plot area by harvesting all the cobs excluding the cobs bordering the plot. The grains were separated from the cob, dried, and weighed. Grain moisture was determined immediately after weighing and subsamples were dried in an oven at 65 °C for 48 hours.

2.7 Calculation of global Warming Potential (GWP) and Greenhouse Gas Intensity (GHGI)

The GWP for all treatments in both seasons was calculated using the following formula [13]:

Net CO_2 eq. emission (GWP) (kg CO_2 eq.ha^{-1}) = {CO_2 (kg ha^{-1}) × 1} + {N_2O (kg ha^{-1}) × 298}

The GHGI was calculated by dividing the GWP for each treatment by the respective grain yield in kg ha^{-1}.

2.7. Data analysis

Statistical analyses of the data were performed using SPSS (version 14), developed IBM statistics. Analysis of variance was carried out to test whether the differences between means were statistically significant. Unless indicated otherwise, differences were considered only when significant at *P*=.05. Tukey's HSD was used to differentiate between the treatment effects.

3. RESULTS AND DISCUSSION

3.1. Temporal trend in nitrous oxide emission

After each fertilizer application, there was a sharp increase in emissions, followed by a gradual decline until the next application. Significant peaks in N_2O emissions aligned closely with fertilizer application dates, indicating that fertilizer application is a major driver of N_2O emissions (Figure 1 (a) and (b)).Initially, all treatments showed relatively low levels of N_2O emissions. After every dose of N application, N_2O flux increased due to availability of substrate for nitrification. Denitrification might have taken place in some anaerobic microsites in the soil, resulting in N_2O flux [14]. This may occur when O_2diffusion is impeded by water, either at the centres of soil aggregates [15] or in saturated regions within a structureless soil [16] or where the local O_2demand is exceptionally high [17]. But, the chances of appreciable N_2Oproduction through denitrification was limited due to high O_2concentration, which is toxic to denitrifiers. Substantial increase in N_2O fluxes was observed afterthe applications of irrigation, which enhanced activities of nitrifiers and denitrifiersin soil.

As expected, the control treatment showed the lowest N_2O emissions throughout the period. Urea-treated plots showed higher N_2O emissions compared to the control. NCU treatment showed moderate emissions, generally lower than Urea but higher than the control. The emissions peaked following fertilizer application but are less pronounced than with Urea. CCU treatment exhibited a similar trend to NCU but with slightly higher emissions. Urea (U) showed the highest peaks in N_2O emissions, suggesting that it may be more prone to volatilization and nitrification-denitrification processes, leading to higher N_2O emissions.Coated urea treatments (NCU and CCU) tend to have moderated peaks, likely due to the controlled release of nitrogen, which helps in reducing the immediate availability of nitrogen for microbial processes that produce N_2O.

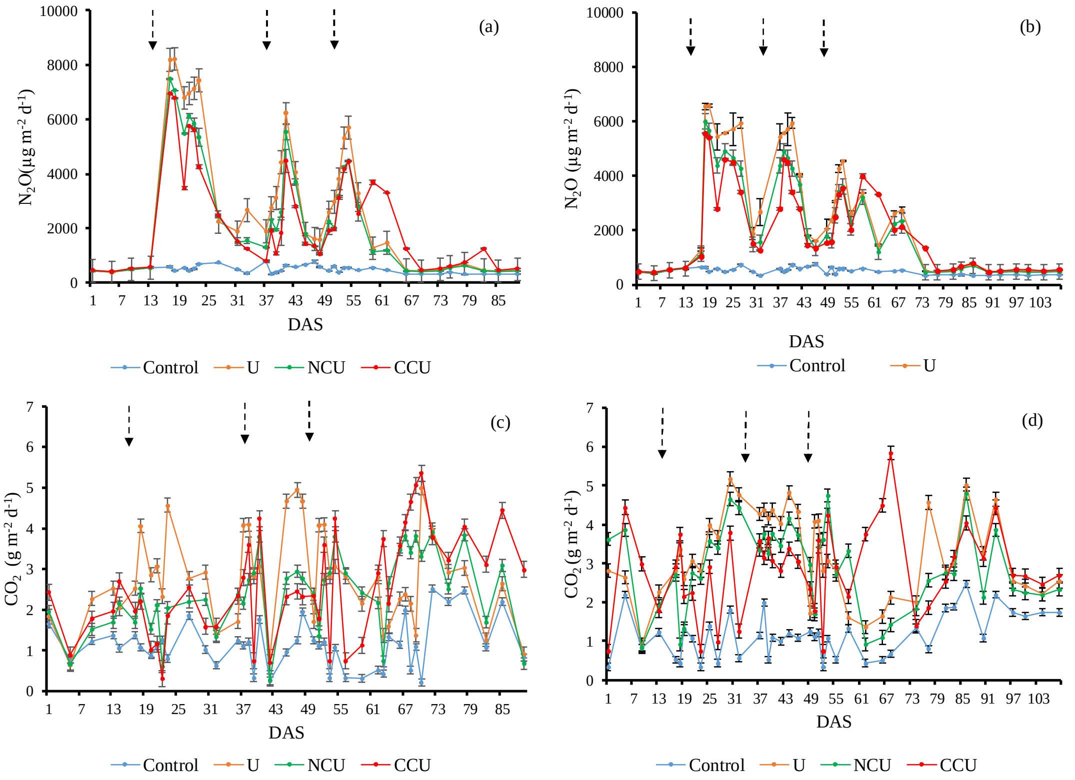

Figure 1 Temporal trend in the emission of greenhouse gases from maize field under various N treatments (a) N_2O (season I) (b) N_2O (season II) (c) CO_2 (season I)(d) CO_2 (season II)

3.2 Temporal trend in carbon dioxide emission

The carbon dioxide emissions from the maize field in both seasons did not show any pronounced trend (Fig. 1 (c) and (d)). During most part of the crop season, the CO_2 emissions from urea and CCU remained higher than the rest of the treatments, owing to the availability of more substrate for microbial respiration. The peaks in CO_2 emissions in all treatments corresponded with the fluctuations in soil moisture, as moisture is one of the influencing factors for microbial respiration.[18] reported thatsoil moisture plays an integral role in determining CO_2 emissions; a prolonged period with deficient or excess water in the soil can cause soil CO_2 emission rates to fall.It has also been reported that soil temperature is the main factor driving variation in soil CO_2 emission rates[19]. Since maize is cultivated during the rainy season, corresponding with fluctuations in soil moisture and temperature in response to rainfall events, it is one of the major reasons for the undulating trend in CO_2 emissions.

3.3. Temporal trend in ammonia emission

In first season (2020), ammonia volatilization in the urea treatment shows there is a sharp increase in ammonia volatilization shortly after the application of fertilizer, followed by a gradual decline over time (Fig. 2 (a)). The peak volatilization occurs shortly after fertilizer application. This is because the applied urea undergoes hydrolysis within the first 3-4 days after fertilizer application. NCU treatment results a delayed peak in volatilization compared to Urea. The peak is lower than that observed with Urea, indicating better retention of nitrogen. Similar to NCU, the CCU treatment shows a delayed and lower peak in ammonia volatilization. The trend is smoother, with less fluctuation compared to Urea and NCU during the first season.

The volatilization trend in the second season for Urea mirrors that of the first season, with a sharp increase post-application followed by a gradual decline (Fig. 2 (b)). However, the overall peak appears slightly lower than in the first season.The NCU treatment in the second season also shows a delayed peak in volatilization, which is again lower than Urea, and the trend is smoother compared to the first season.In the second season, CCU continues to demonstrate the lowest and most stable ammonia volatilization trend among the fertilized treatments, with a smooth and delayed peak similar to the first season. The lowering of ammonia emissions from CCU is an indication of its urease inhibitory activity. The inhibition of soil urease leads to an increase in the duration of complete hydrolysis of urea, delaying all the further transformation processes like ammonia oxidation, nitrification and denitrification. This results in the smoother curve of the CCU treatments.

The general trend for all treatments in the second season shows slightly lower peak ammonia volatilization levels compared to the first season.This is because there was more rainfall during the second season, which caused less volatilization and more leaching losses. [20] have observed that the occurrence of heavy rainfall immediately after urea application reduces ammonia volatilization. To reduce NH_3 volatilization from Urea, it is essential to apply the fertilizer when soil and air temperatures are cool, the soil surface is moderately dry, or when rain occurs soon after application. Rain helps in incorporating the fertilizer into the soil shortly after application and can also significantly reduce or prevent NH_3 volatilization[21].

The NCU and CCU treatments demonstrate more stability with smoother and lower peaks across both seasons compared to Urea, with CCU consistently showing the lowest peaks.This result highlights the benefits of using Neem Oil Coated Urea and Cashew Nut Shell Liquid Coated Urea over conventional Urea, as these treatments reduce ammonia volatilization, improving nitrogen use efficiency in maize cultivation.

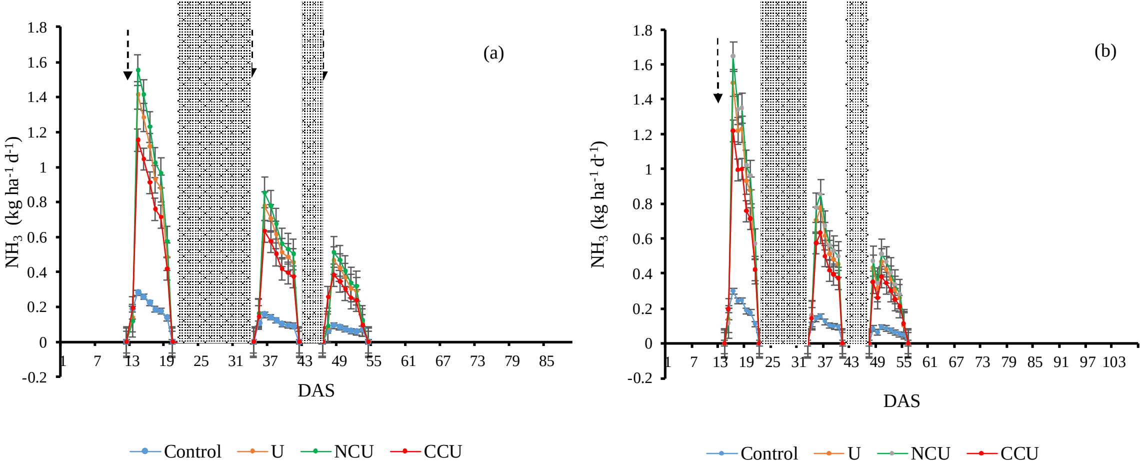

Figure 2 Ammonia volatilization from maize (a) first season and (b) second season under different nitrogen fertilizer treatments. The vertical arrows depict the dates of fertilizer application. The blue shaded areas indicate the time period where ammonia volatilization was below the limits of detection.

3.3. Cumulative ammonia emissions

In first season, Urea showed the highest cumulative ammonia emissions among all treatments (Fig. 3).NCU resulted in significantly lower cumulative ammonia emissions compared to Urea, showing its effectiveness in reducing ammonia losses.CCU also showed lower emissions than Urea, similar to NCU, indicating a significant reduction compared to Urea and NCU.

Coming to second season, the Urea treatment continued to exhibit the highest emissions, though slightly lower than in the first season. It was still significantly higher than the other treatments.NCU showed a similar trend as in the first season, with emissions lower than Urea but higher than CCU.In the second season, CCU showed the lowest emissions among the fertilized treatments, slightly lower than in the first season.

The Urea treatment consistently resulted in the highest cumulative ammonia emissions in both seasons, although there was a slight decrease from 2020 to 2021.Both NCU and CCU were effective in reducing cumulative ammonia emissions compared to Urea. However, CCU consistently outperformed NCU, resulting in the lowest emissions among the treated groups in both seasons.This interpretation highlights the advantage of using Neem Oil Coated Urea and Cashew Nut Shell Liquid Coated Urea over conventional Urea. Both treatments significantly reduce ammonia emissions, with CCU showing the best performance across both seasons.Anacardic acid has been shown to form lipophilic metal derivatives with an unusually high degree of selectivity.Synthesis of linear, lipophilic metal derivatives with a high degree of selectivity (particularly to the important transition metal ions, Fe^{2+} and Cu^{2+}, and, to a lesser extent, Zn^{2+}) may explain the wide spectrum of biological activity for anacardic acid as a inhibitor of metal-dependent enzymes. It can bind to the nickel ions present in soil urease enzyme, thereby inhibiting it. Pharmacological studies involving anacardic acid have shown complete urease inhibition at a concentration of 0.12mg/ml [5].

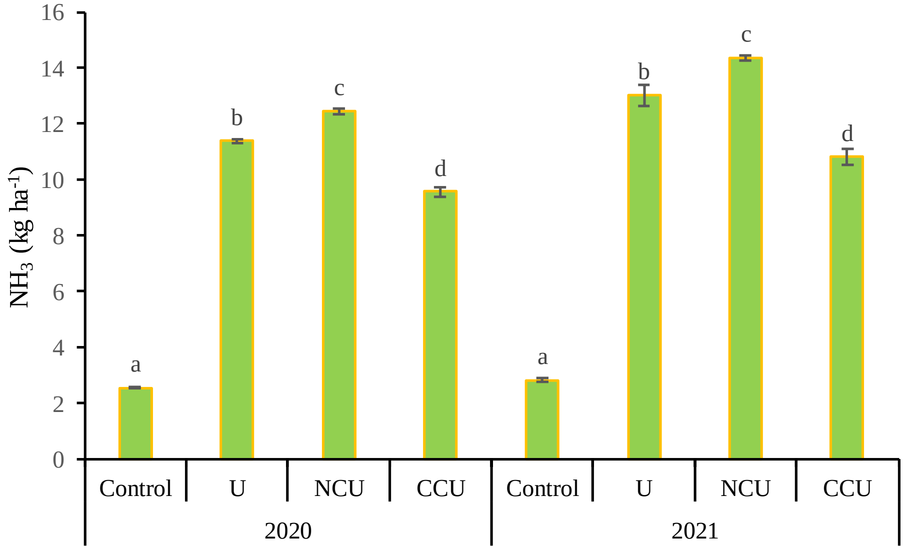

Figure 3 Cumulative ammonia emissions in two seasons of Maize under different N fertilizer treatments. The same letters over the bars indicate that the two values are not significantly different.

Table 2: Cumulative greenhouse gas emissions, GWP and GHGI

Treatment	CO_2 emission (kg ha^{-1})	N_2O emission (kg ha^{-1})	GWP(kg CO_2 eq. ha^{-1})	Yield (kg ha^{-1})	GHGI (kg CO_2 eq. kg^{-1} yield)
Season I					
C	1068.78±12.39^{a}	0.361±0.0009^{a}	1176.4±12.28^{a}	2983.29±122.67^{a}	0.397±0.016^{a}
U	2312.33±9.14^{b}	1.942±0.0240^{b}	2890.9±9.51^{b}	6680.57±94.26^{b}	0.433±0.006^{a}
NCU	2026.32±9.61^{c}	1.615±0.0149^{c}	2507.5±11.88^{c}	7311.50±84.08^{c}	0.343±0.003^{b}
CCU	2219.66±16.96^{d}	1.661±0.0140^{c}	2714.6±15.94^{d}	6833.63±87.86^{b}	0.398±0.006^{a}
HSD (P=0.05)	50.31	0.064	51.08	0.398	0.038
Season II					
C	1095.00±7.50^{a}	0.513±0.0001^{a}	1247.9±7.51^{a}	2853.57±96.83^{a}	0.439±0.014^{a}
U	2852.20±11.27^{b}	2.246±0.0207^{b}	3521.4±10.86^{b}	5213.92±85.28^{b}	0.676±0.011^{b}
NCU	2549.50±27.55^{c}	1.884±0.0189^{c}	3111.0±26.17^{c}	5415.91±74.06^{b}	0.575±0.012^{c}
CCU	2592.03±25.96^{c}	1.859±0.0108^{c}	3146.1±27.16^{c}	5466.17±34.23^{b}	0.576±0.007^{c}
HSD (P=0.05)	81.40	0.061	80.92	0.309	0.046

Both cumulative CO_2 and N_2O emissions exhibited significant variation among the treatments in both seasons, as depicted in Table 2. The plots fertilized with prilled urea showed the highest CO_2 and N_2O emissions, and consequently the highest GWP. Both NCU and CCU emitted significantly lower levels of CO_2 and N_2O in both seasons compared to urea. There was no significant difference between NCU and CCU in their cumulative N_2O emissions in both years. On an average, NCU plots emitted 11.39% less CO_2 and 16.47% less N_2O when compared to urea, amounting to a net GWP decrease of 12.37%. CCU treated plots exhibited a net GWP decrease of 8.59% compared to urea and an increase of 4.13% compared to NCU. The increased GWP was mostly due to the increased CO_2 emissions.

In the first season, NCU had significantly higher grain yield than the other treatments, while CCU treatment gave yields slightly higher than that of urea, but both yields were statistically similar. The possible reason for yield enhancement might be due to continuous and steady supply of N into the soil by coated fertilizers to meet the required nutrient for physiological processes, which in turn improved grain yield [22].

Interestingly, in the second season, the yields of the three N fertilizer treatments were not significantly different. This may be attributed to the increased rainfall and associated N losses in the second season. GHGI was lower in the case of CCU as well as NCU compared to urea in both seasons, owing to their significantly lower GHG emissions. This proves the efficacy of CCU as a controlled release N fertilizer that can reduce N losses from agricultural soils and improve the application efficiency of N fertilizers by reducing ammonia volatilization losses. That said, since anacardic acid is more selective towards Fe^{2+}, Cu^{2+} and Zn^{2+} when comparedto Ni^{2+}, CNSL coated urea may not be suitable for acidic soils with a predominance ofFe^{2+}ions. The suitability of this urease inhibitor in different soil types and agroclimatic zones needs to be evaluated with further studies.

4. CONCLUSION

The use of coated urea (NCU and CCU) effectively reduces the N_2O emissions compared to uncoated Urea, providing a potential strategy for reducing the environmental impact of nitrogen fertilizers in agriculture.

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