*Pak. J. Bot.*, 57(1): DOI: http://dx.doi.org/10.30848/PJB2025-1(29)

# OPTIMIZING NEEM OIL COATING THICKNESS FOR THE DEVELOPMENT OF SLOW-RELEASE UREA FERTILIZER TO ENHANCE WHEAT GROWTH AND YIELD

AMIR AZIZ[1], MUKKRAM ALI TAHIR[1], NOOR-US-SABAH[1], AMBER RAZA[2], ZAHOOR AHMAD[3*], ABEER HASHEM[4], GRACIELA DOLORES AVILA-QUEZADA[5] AND ELSAYED FATHI ABD_ALLAH[6]

[1]*Department of Soil and Environmental Sciences, College of Agriculture, University of Sargodha, Pakistan;*
[2]*Department of Botany, Government Sadiq College Women University, Bahawalpur, Pakistan;*
[3]*University of Central Punjab, Constituent Punjab College Yazman Road Bahawalpur, 63100, Pakistan;*
[4]*Botany and Microbiology Department, College of Science, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia;*
[5]*Facultad de Ciencias Agrotecnológicas, Universidad Autónoma de Chihuahua, 31350, Chihuahua, Chihuahua, México;*
[6]*Plant Production Department, College of Food and Agricultural Sciences, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia.*
**Corresponding author's email: zahoorahmadbwp@gmail.com, Ph.: +92-300-6878455.*

**Abstract**

This study aimed to investigate how slow-release fertilizers affect nitrate leaching, soil profile, and yield of wheat. In the current climate, there is growing interest in developing new controlled-release fertilizers based on eco-friendly raw materials. Neem oil coating layers on urea granules were compared and different parameters were investigated in the present study. When comparing several coating layers, single coatings showed a higher release than double and triple coatings earlier in the fertilizer application process, indicating a less efficient coating. A triple coating significantly reduced the amount of release that might not meet plant requirements. In the second study, physiological parameters and crop yield were compared between neem oil-coated fertilizers (75, 50%, and 25%) and untreated fertilizers (Control). Results of the pot study showed that a reduced dose of fertilizer (75%) favored a better response in photosynthetic parameters and nutritional status in wheat plants. There was a significant increase in the yield of grain weight and number of grains with the neem oil coating, since it was 75% more effective than the uncoated coating. Results showed that the neem oil-coated urea fertilizer improved crop management and reduced N losses, which are usually caused by conventional fertilizers. A completely randomized design was used for all laboratory and pot experiments. Hence, natural neem oil as a coating material has the potential to boost growth and yield as well as control negative environmental effects.

**Key words:** Neem oil, Urea, Dose optimization, Slow-release fertilizer, Wheat.

## Introduction

The application of synthetic nitrogen (N) fertilizer to cropland alters greenhouse gas emissions, water quality, global nutrient balance and feedback to the climate system, in addition to increasing agricultural production. However, due to the lack of geographic data on fertilizer inputs, existing Earth system and land surface modeling studies must ignore or use oversimplified data (e.g., static, spatially uniform fertilizer use) to characterize agricultural N inputs over decades or centuries (Lu & Tian, 2017). Nitrogen fertilizer is widely used to increase crop production in arable land (Vitousek *et al*., 2009; Martinez-Feria *et al*., 2018). N supply over the crop's demand leads to N losses as NO3--N leaching (Valkama *et al*., 2016; Hansen *et al*., 2019; Umar *et al*., 2020), NH3 volatilization (Pfromm, 2017; Ying *et al*., 2019), N2O/NO/N2 emission (Loick *et al*., 2016; Huddell *et al*., 2020), resulting in non-point-source pollution (Sha *et al*., 2020) global warming (Ogle *et al*., 2014) and other negative environmental impacts. Among these losses, major N loss is via ammonia volatilization (Shaviv & Mikkelsen, 1993; Frame, 2017; Ashraf *et al*., 2019). Therefore, it is crucial to improve strategies to reduce negative environmental impacts while maintaining crop yields (Suter *et al*., 2020).

Wheat (*Triticum aestivum* L.) is the most widely grown crop in temperate climates and is used for both human and cattle feed. Wheat provides the human diet with important vitamins, minerals, and amino acids, as well as beneficial dietary fiber and phytochemicals, which are particularly abundant in whole-grain products (Shewry, 2009). Wheat production feeds millions of people around the world and is largely reliant on adequate nitrogen supply.

Nitrogen is a major nutrient that restricts the productivity of wheat (Fradgley *et al*., 2021). There is also a lot of variation in how wheat cultivars acquire and use nitrogen to produce higher yields (Belete *et al*., 2018). Thus, applying the correct rate of nitrogen fertilizer is regarded as a main means of raising wheat grain yield, and enhancing N uptake and use efficiency, but N-losses due to volatilization leads to reduced yield and nutrient-use efficiency of wheat (Fageria, 2014). To enhance nitrogen-use efficiency (NUE) and minimize N losses effectively, several strategies have been employed in recent years (Langholtz *et al*., 2021). Use of urease and nitrification inhibitors is an effective technique in minimizing the N losses (Byrne *et al*., 2020), but urease and nitrification inhibitors are too expensive for common farmers (Zaman *et al*., 2013). Breeding approaches are also employed for enhancing nitrogen fixation but this is a time-consuming process Lindström and Mousavi, 2020). To avoid the negative environmental consequences of urea application, it is recommended to use controlled-release urea (CRU). At soil pH 6.0, CRU effectively reduces $N_2O$ emissions and $NH_3$ volatilization (Zhang *et al*., 2019). Blending urea reduces the ammonia volatilization losses very effectively up to 17–20% as compared to the uncoated urea (Zhang *et al*., 2021). Polymer coating of urea is a cheaper and effective technique to lower the N losses, and for enhancing the nitrogen-use efficiency (Wang *et al*., 2015). Polymer coated urea significantly enhanced the corn yield and nitrogen-use efficiency by lowering the N losses (Xie *et al*., 2020).

Furthermore, during the application of slow release fertilizer the loss of nitrogen is reduced (Joshi *et al.,* 2014). In some countries, this is the traditional method to mix the urea with neem cake to increase the nitrogen use efficiency (Singh *et al*., 2019). Urea is coated by neem oil and reduces the N losses and N is slowly released to crop throughout its life cycle (Thind *et al*., 2010). Although it had been established long ago that neem products, when applied along with urea can enhance NUE in crops (Meena *et al.,* 2018). Therefore, research investigations based on science-based experiments were critically analyzed to better understand the agronomic benefits of different coating layers and amounts of neem oil-coated urea compared to ordinary urea.

## Material and Methods

**Experimental location and treatments (coating on urea granules):** A laboratory and pot experiment was conducted at Wahdat Ingegrated Agro Farm, Sargodha to optimize the use of neem oil for coating on urea with the aim of enhancing nitrogen use efficiency of wheat crop. Concentrations of neem oil (1.0%) were made in acetone and coated on urea granules with the help of coating machine (Fig. 1). Controlled conditions were maintained to avoid any contamination, during these activities under laboratory conditions. Coated urea was dried under laboratory conditions and stored at room temperature (25°C).

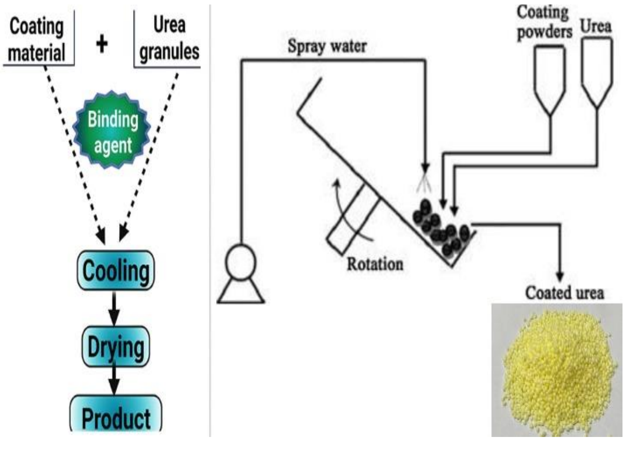


Fig. 1. Flow chart diagram coating on urea granules with the help of urea coating machine.

**Soil analysis:** Soil to be used in the laboratory and pot experiments was bulk sampled from the top layer 15cm layer. Composite sample was pre-analyzed for various physio-chemical parameters at departmental Laboratory. The details of the tested soil sample are given in table:

**Table 1. Physico-chemical properties of soil taken for experiments.**

| Properties | Units | Readings |
|---|---|---|
| Sand | % | 50.54 |
| Silt | % | 27.36 |
| Clay | % | 22.10 |
| Textural class | - | Sandy loam |
| Moisture percentage | % | 30 |
| ECe | dS $m^{-1}$ | 1.85 |
| pH | - | 7.67 |
| CEC | meq/100 g | 4.27 |
| O.M | % | 0.50 |
| K | mg $kg^{-1}$ soil | 106 |
| N | % | 0.07 |
| P | mg $kg^{-1}$ soil | 6.5 |

This study was conducted in the laboratory to check the effect of different number of coatings of neem oil (1.0%) on urea fertilizer. The experiment was designed according to completely randomized design CRD. Each treatment was replicated three times. The treatment plan was consisted of:

a) Uncoated urea (Control)
b) Number of coatings (uncoated, single, double and triple)

Four treatment sets were run for the determination of $NH_4^+$ and $NO_3^-$ release at 10, 20 and 30 days intervals.

A pot experiment was conducted in the Wahdat Ingegrated Agro Farm Sargodha, to evaluate the effect of neem oil coated urea rates on growth, yield and nitrogen use efficiency of wheat. Best number of coating layers (Double) was selected from Laboratory experiment. The treatment plan was comprised of:

a) Control (without N fertilizer)
b) Uncoated urea (Control)
c) Fertilizer rates (75, 50 and 25% of recommended rate of neem oil coated urea in comparison with recommended rate of uncoated urea)
d) Wheat varieties: Barani (Barani-2017) and Irrigated (Akbar-2019)

Pots were filled with pre-analyzed soil at the rate of 10 kg per pot. Pots were arranged according to completely randomized design (CRD) for 5 treatments and each treatment with three replications. Tap water was applied to all pots to achieve field capacity level. At field capacity 5 seeds of wheat varieties "Barani-2017 and Akbar-2019" were sown in pots. After germination two plants were maintained in each pot by manual thinning. Urea fertilizer was used as N source while single super phosphate (SSP) and sulphate of potash (SOP**)** were used as P and K sources at the rate of 120: 90: 60 Kg $ha^{-1}$ (N: P: K). All P and K was applied at sowing, while urea (in coated and uncoated form) was applied with first irrigation. Uncoated urea was applied at recommended rate, while coated fertilizers were applied at 75, 50 and 25% of recommended rate of N. All other agronomic practices for pest control and growth requirements were adopted as and when needed.

**Measurement of ammonium, nitrate and cumulative nitrogen:** Different number of coatings of neem oil on urea fertilizer was carried out at Laboratory. Neem oil was coated in single, double and triple layers on urea. Field capacity moisture was maintained each after 24 hours and $NH_4^+$ and $NO_3^-$ were measured after (10, 20, 30) days of incubation through indophenol blue method (Phenol and hypochlorite react with ammonia to form indophenol blue that is propotional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside and measured colorimetrically) and phenol disulphonic acid method (Nitrate reacts with phenol disulfonic acid and produces a nitro derivatives which in alkaline solution develops yellow colour due to rearrangement of its structure) respectively (Keeney & Nelson, 1989). Calculation for cumulative N was measured by sum of ammonium and nitrate concentration. The collected data were analyzed following Fisher's analysis of variance for CRD and LSD test was used for mean comparison (Steel *et al*., 1997).

**Evaluation of plant growth and yield of wheat:** At the crop's physiological maturity, the chlorophyll contents were recorded using a SPAD meter (Estimation of chlorophyll contents by correlations between SPAD-502 meter and chroma meter in butterhead lettuce). Plant height and spike length were measured using a meter rod. After harvesting of wheat crop, number of fertile, unfertile and total tillers per pot were counted and yield components (fresh and dry weight of shoot, 1000 grains weight, straw yield and biological yield weighed using a weighing balance) of wheat were noted. Number of grains per spoke and spikelets per spike were noted. Harvest index % was calculated.

$$\text{Harvest index (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

**Chemical analysis of soil and plant:** Several soil properties were measured to characterize soil fertility in both experiments. pH and EC were determined in a 1/5 (w/v) aqueous soil extract by shaking and filtration. pH was measured by a pH meter (Crison mod.2001, Barcelona, Spain) and EC with a Conductivity meter (Crison micro CM2200, Barcelona, Spain). Total and organic soil C (SOC) and total N (N) were determined by combustion gas chromatography in a Flash EA 1112 Thermo Finnigan (Franklin, MA, USA) elemental analyzer after eliminating carbonate by acid digestion with HCl. The total nutrient contents (P, K, Ca, Mg, Cu, Fe, K, Mg, Mn, and Zn) were extracted by aqua regia digestion (3:1, v/v, HCl/HNO3) and determined by ICP-AES (Thermo Elemental Iris Intrepid II XDL, Franklin, MA, USA).

Chemical analysis for N concentrations in the grain, straw and roots were performed at departmental laboratory. Grain, straw and root samples were digested following method proposed by Wolf (1986) by using sulfuric acid and hydrogen peroxide. Nitrogen concentration from digested plant samples was estimated following the Kjeldahl method (Jackson, 1982).

## Statistical analysis

Data collected from lab and pot experiments was analyzed using completely randomized design (CRD) for Fisher's analysis of variance (Steel *et al*., 1997). Treatment means were compared through LSD test.

## Results

**Ammonium ion concentration in soil at different time intervals (10, 20, 30):** Table 2 depicted that ammonium release from uncoated urea was maximum at first interval, but it was reduced enormously at latter intervals (after 20 and 30 days of interval). While all coating layers with neem oil (single, double, triple) lowered the ammonium release at first interval in comparison to uncoated urea, but it was significantly increased at second interval. At third interval, though ammonium release was significantly lower than at second interval but it was still higher than release at first interval. However, maximum ammonium release was given by double coating of neem oil that was 1563 mg $kg^{-1}$. Among number of coating layers, double coating released maximum ammonium at second and third interval for all coating layers.

**Nitrate ion concentration at different time intervals (10, 20, 30):** The nitrate release was significantly lower in coated treatments than uncoated treatment at first and second interval. At third interval nitrate concentration from coated treatments was a little increased than uncoated treatment (Table 3). At first interval maximum nitrate concentration (1162.0 mg $kg^{-1}$) was found in soil treated with uncoated urea, while minimum was found with negative control (1.0 mg $kg^{-1}$). Single coating released more nitrate in the soil than double and triple coating for neem oil at first interval. But at third interval, double layer coating of neem oil higher nitrate release than single and triple layer coating.

**Cumulative N concentration in soil at different time intervals (10, 20, 30):** Cumulative nitrogen showed a consistent decline in negative control and uncoated urea treatment, while in coated fertilizers cumulative nitrogen concentration was increased up to second interval then slightly lowered at third interval but the value were still higher than that of first interval, as indicated in Table 4. At first interval maximum cumulative N release was seen for uncoated urea (2618.0 mg $kg^{-1}$), while among coated treatments double coating of neem oil induced highest cumulative N release that was 714 mg $kg^{-1}$. At second interval maximum release of cumulative N was given by double coating of neem oil (1.0%). It was noted that cumulative N release was much consistent from double layer coating of all number of coating layers. As highest cumulative N release (2146.0 mg $kg^{-1}$) was given by double coating of neem oil at 20 days interval.

**Plant height:** Plant height is considered a fundamental morphological character which is genetically controlled but also influenced by nutrients, water and environmental stress. Plant height is a best indicator of vegetative growth of plant, more the vegetative growth more will be the plant height. Maximum plant height (82.95 cm) was seen where 75% neem coated urea as compared to recommended rate of commercial urea was applied followed by uncoated recommended urea (76.85 cm) in irrigated wheat variety "Akbar-2019". Reduced rates of all types of coated urea also gave excellent response; even 25% of recommended rate neem oil coated urea gave plant height (69.75 cm) best to negative control (Fig. 2). Furthermore, in barani wheat variety "Barani-2017" 75% neem oil coated urea gave more plant height (78.87 cm) in comparison to all other treatments.

**Table 2. Effect of coating layers on ammonium concentrations in the soil at different time intervals (10, 20, 30).**

| Fertilizer sources | Number of coating layers | Ammonium (mg $kg^{-1}$) Time interval (days) | | |
|---|---|---|---|---|
| | | 10 days | 20 days | 30 days |
| Negative control | No fertilizer, No coating | 1.7 K | 0.7 K | 0.4 K |
| Positive control | Uncoated | 1456 C | 432 H | 238 J |
| Neem oil coated urea | Single | 457 G | 1496 B | 957 DE |
| | Double | 453 G | 1563 A | 965 EF |
| | Triple | 346 I | 1178 D | 882 F |

**Table 3. Effect of coating layers on nitrate concentrations in the soil at different time intervals (10, 20, 30).**

| Fertilizer sources | Number of coating layers | Nitrate (mg $kg^{-1}$) Time interval (days) | | |
|---|---|---|---|---|
| | | 10 days | 20 days | 30 days |
| Negative control | No fertilizer, No coating | 1.0 H | 0.6 H | 0.5 H |
| Positive control | Uncoated | 1162 A | 843 B | 307 EF |
| Neem oil coated urea | Single | 264 F | 431 E | 663 C |
| | Double | 261 F | 583 D | 671 C |
| | Triple | 243 G | 424 E | 602 CD |

**Table 4. Effect of coating layers on cumulative N concentrations in the soil at different time intervals (10, 20, 30).**

| Fertilizer sources | Number of coating layers | Cumulative nitrogen (mg $kg^{-1}$) Time interval (days) | | |
|---|---|---|---|---|
| | | 10 days | 20 days | 30 days |
| Negative control | No fertilizer, No coating | 2.7 J | 1.3 J | 0.9 J |
| Positive control | Uncoated | 2618 A | 1275 F | 545 I |
| Neem oil coated urea | Single | 721 G | 1927 BC | 1620 CD |
| | Double | 714 G | 2146 B | 1636 C |
| | Triple | 589 H | 1602 D | 1484 E |

**Chlorophyll content:** Chlorophyll is an important photosynthetic pigment to the plant, largely determining photosynthetic capacity and hence agronomic plant growth. Figure 3 depicted that minimum chlorophyll contents were found where no nitrogen (N) fertilizer was applied, while all rates of N fertilizers significantly improved the chlorophyll contents in both varieties (Irrigated and Barani) wheat plants. Among neem oil coated urea treatment, 75% rate of recommended dose induced maximum chlorophyll contents (46 and 43). Even the reduced rate (50% of recommended) of neem oil coated urea induced greater chlorophyll contents (40 and 38) in wheat plants than control.

**Fresh weight of shoot:** Shoot fresh weight (SFW) is one of the parameters, used to estimate the total plant biomass yield in wheat. Shoot fresh weight was increased in the wheat of plant treated with all levels of coated urea (Fig. 4). Even reduced rates (75, 50 and 25% of recommended rate) of all coated urea fertilizer induced higher chlorophyll contents than recommended rate of uncoated urea indicating net saving of urea fertilizer up to 25 to 50%. However, maximum Shoot fresh weight was observed in the treatment of neem oil coated urea 75% (2.06 and 1.86 g in both wheat varieties) followed by recommended rate of uncoated urea (1.81 and 1.71). Control treatment induced Shoot fresh weights up to (1.53 and 1.37 g) that were lower than all other treatments.

**Dry weight of shoot:** The conventional means of determining shoot dry weight (SDW) is the measurement of oven-dried samples. In this method, tissue is harvested and dried, and then shoot dry weight is measured. Shoot dry weight was increased in the wheat of plant treated with all levels of coated urea (Fig. 5). Even reduced rates (75, 50 and 25% of recommended rate) of all coated urea fertilizer induced higher chlorophyll contents than recommended rate of uncoated urea indicating net saving of urea fertilizer up to 25 to 50%. However, maximum Shoot dry weight was observed in the treatment of neem oil coated urea 75% (0.42 and 0.38 g in both wheat varieties) followed by recommended rate of uncoated urea (0.40 and 0.36). Control treatment induced Shoot dry weights up to (0.27 and 0.24 g) that were lower than all other treatments.

**Spike length:** Spike length is a main character of variety which is much influenced by water and nutrients. Results regarding spike length (cm) of wheat as affected by the application of different levels of coated urea. It is clear from the data that spike length was significantly affected by the coated urea levels. The interaction between coated urea fertilizer and their levels also provided a significant effect on the spike length. Coated urea 75% showed maximum spike length (12.33 cm and 11.29 cm in both varieties) as compared to other levels of coated urea. The second most spike length (11.50 cm and 10.74 cm) was recorded in uncoated urea fertilizer. Minimum spike length (8.33 cm and 7.50 cm) was found in control treatment where N fertilizer was not applied (Fig. 6).

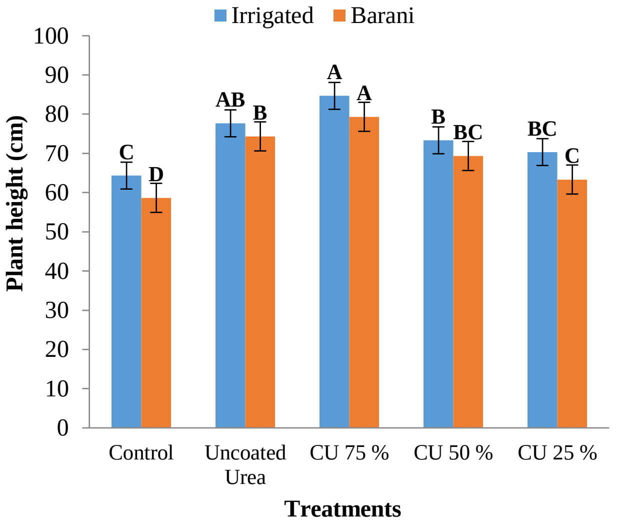


Fig. 2. Impact of different rates of neem oil coated urea on plant height (cm) of wheat.

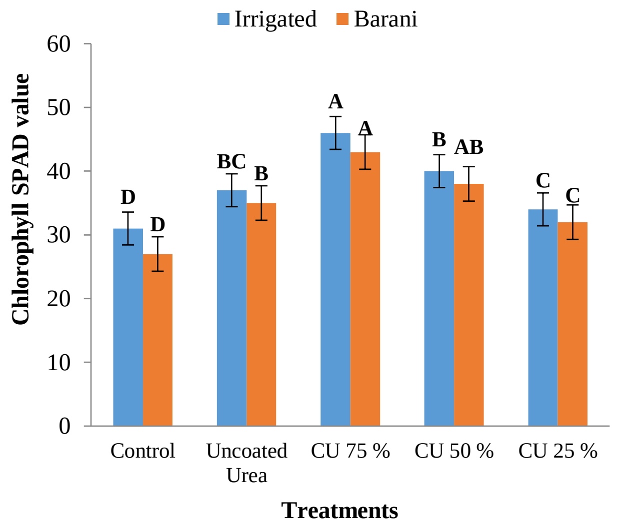


Fig. 3. Impact of different rates of neem oil coated urea on chlorophyll content (SPAD) of wheat.

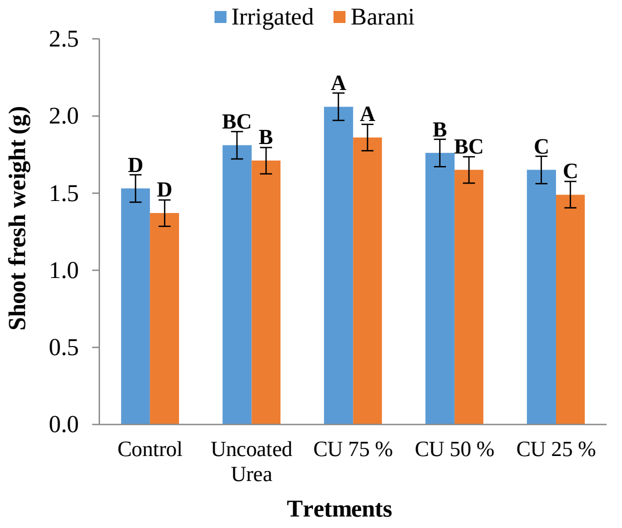


Fig. 4. Impact of different rates of neem oil coated urea on shoot fresh weight (g) of wheat.

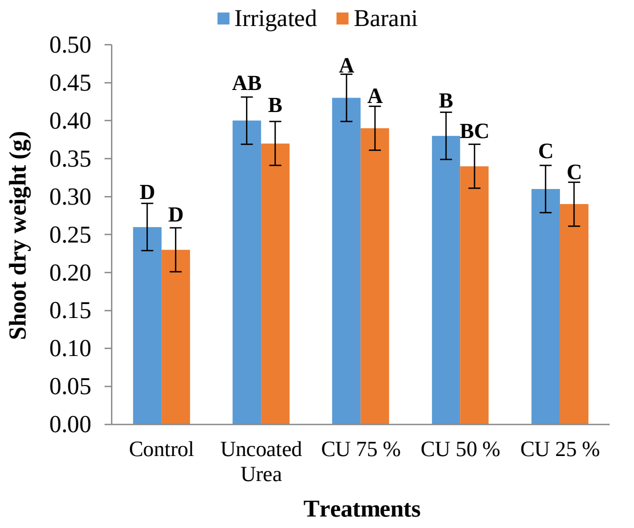


Fig. 5. Impact of different rates of neem oil coated urea on shoot dry weight (g) of wheat.

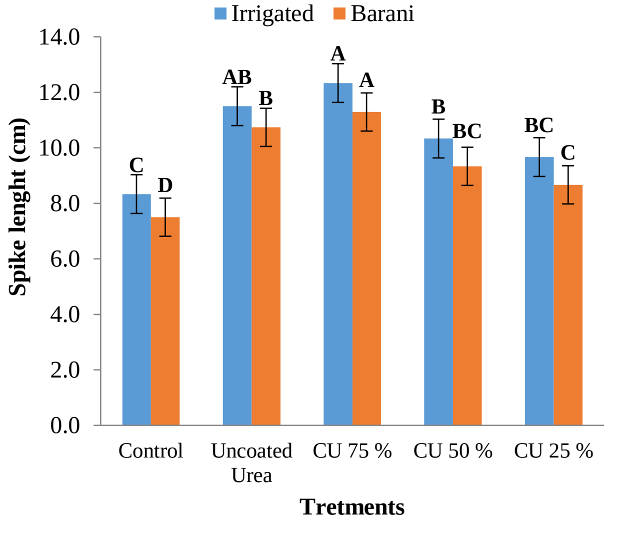


Fig. 6. Impact of different rates of neem oil coated urea on spike length (cm) of wheat.

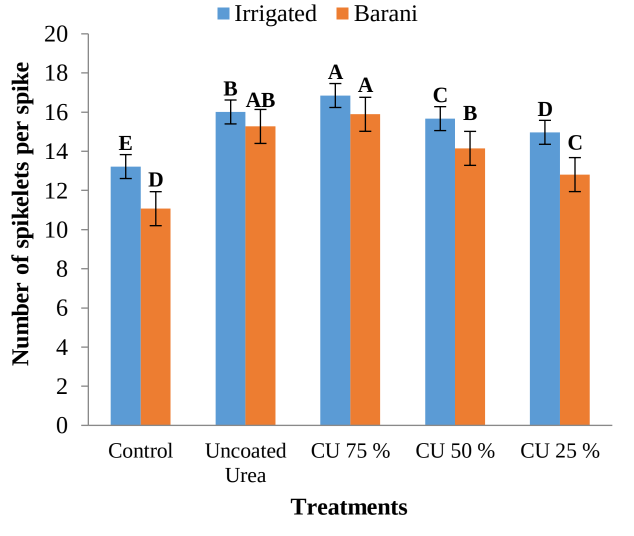


Fig. 7. Impact of different rates of neem oil coated urea on spikelet's per spike of wheat.

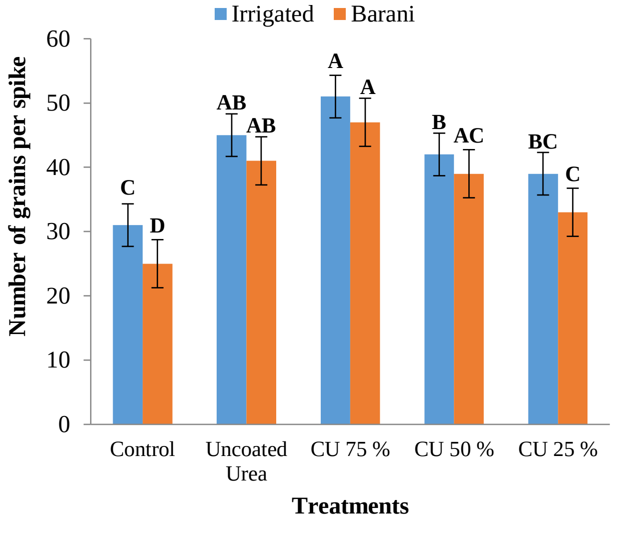


Fig. 8. Impact of different rates of neem oil coated urea on number of grains per spike of wheat.

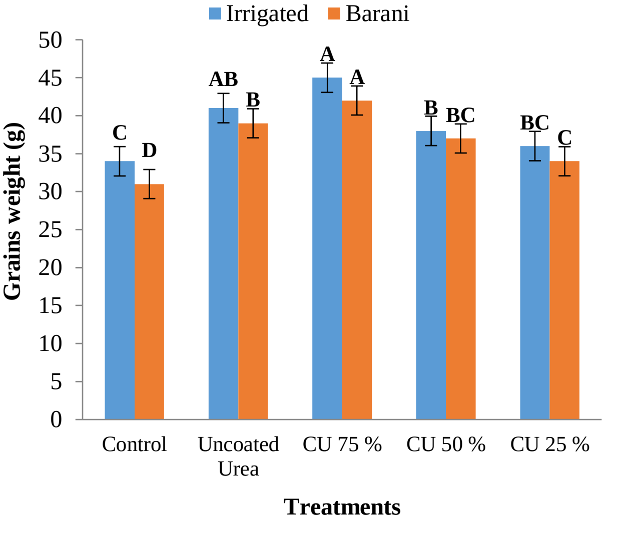


Fig. 9. Impact of different rates of neem oil coated urea on 1000 grains weight (g) of wheat.

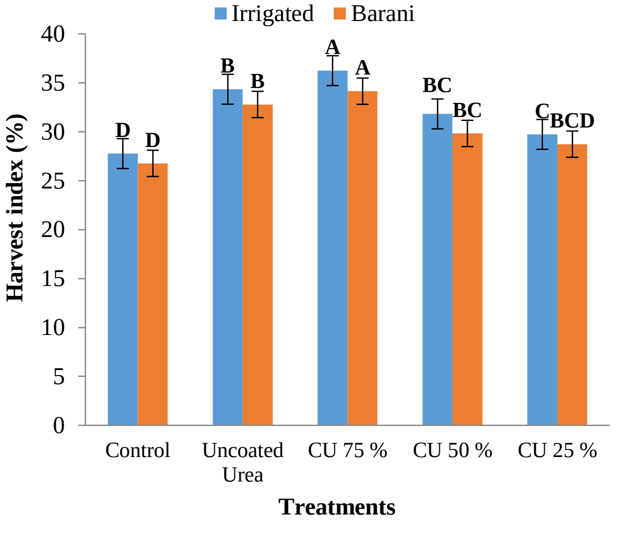


Fig. 10. Impact of different rates of neem oil coated urea on harvest index (%) of wheat.

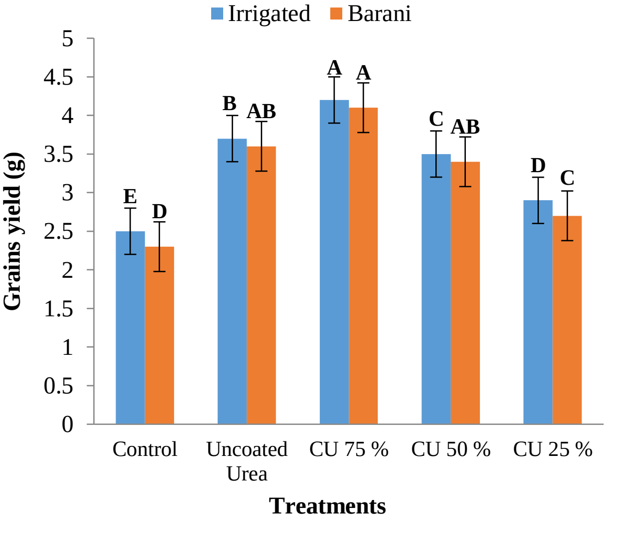


Fig. 11. Impact of different rates of neem oil coated urea on grain yield per plant (g) of wheat.

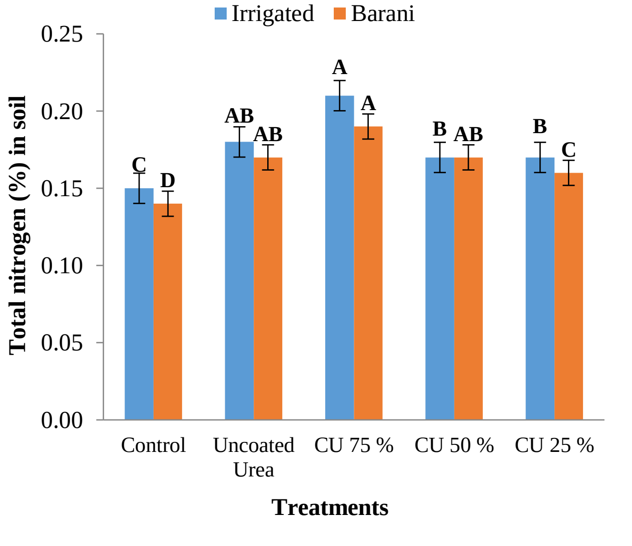


Fig. 12. Impact of different rates of neem oil coated urea on total nitrogen concentration (%) in soil.

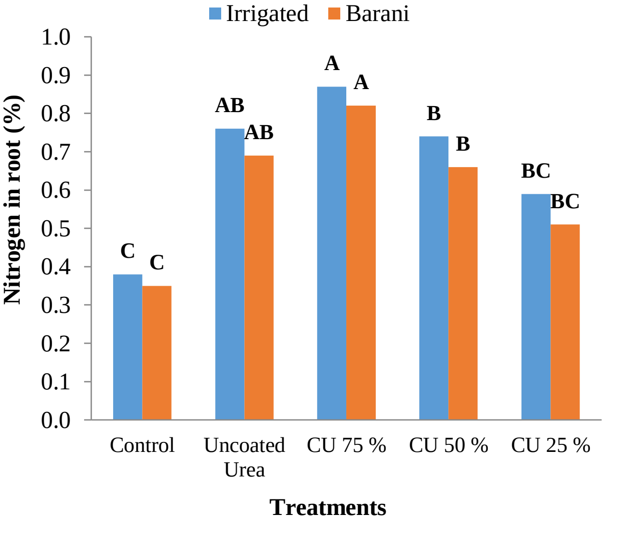


Fig. 13. Impact of different rates of neem oil coated urea on nitrogen concentration (%) in root.

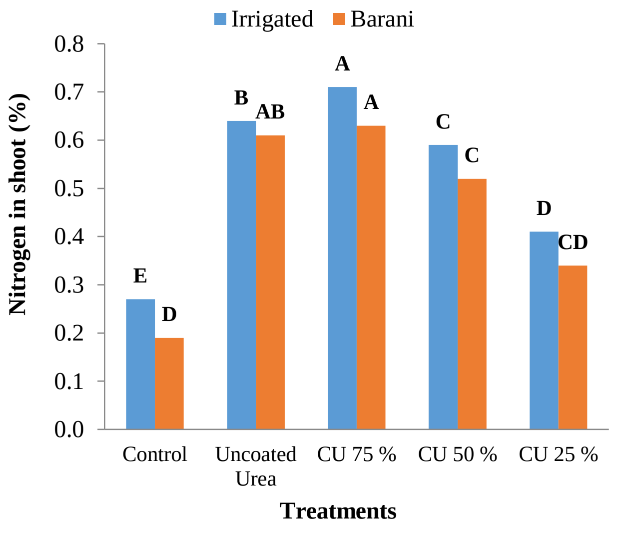


Fig. 14. Impact of different rates of neem oil coated urea on nitrogen concentration (%) in shoot.

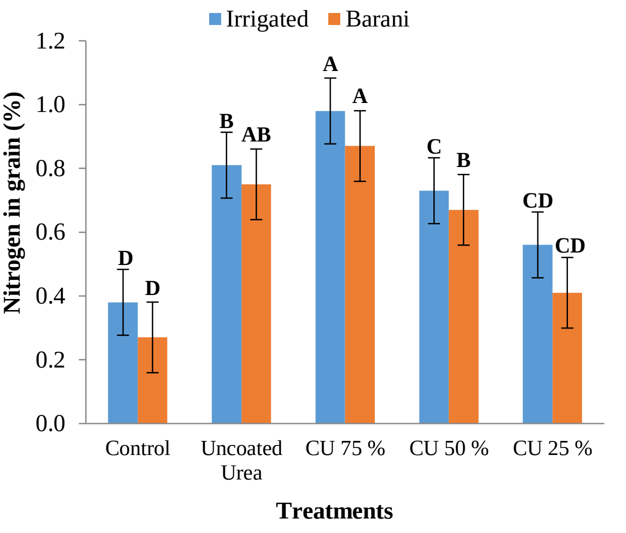


Fig. 15. Impact of different rates of neem oil coated urea on nitrogen concentration (%) in grains of wheat.

**Spikelet's per spike:** Figure 7 depicted the highest spikelets per spike (16.85 and 15.89 in both varieties) in the treatment of neem oil coated urea 75 % among all rates of different levels of coated urea and uncoated urea. However, reduced rate at 50 and 25% of recommended rate neem oil coated urea produced the high spikelets per spike among the control treatment in both varieties. However, minimum spikelets per spike (13.22 and 11.07) were found in control treatment where N fertilizer was not applied.

**Grains per spike:** Number of grains per spike is an important parameter which has a prime position to contributing the grain yield. More the number of grains per spike more will be grain yield. Trend for number of grains per spike minimum grain yield was given by control where no N fertilizer was applied that was 31 per spike in irrigated and 25 per spike in barani wheat variety. Uncoated urea at recommended produced 45 and 41 grains per spike in both varieties respectively that was greater than 50 and 25% of recommended neem oil coated urea (Fig. 8). However, 75% of recommended rates of neem oil coated urea significantly improved the number of grains per spike than recommended rate of uncoated urea in both verities.

**1000 grains weight:** Minimum 1000 grains weight was given by control where no N fertilizer was applied that was 34 g and 31 g in irrigated and barani wheat varieties respectively (Fig. 9). Uncoated urea at recommended produced 41 g and 39 g grain yield that was greater than 50 and 25% of neem oil coated urea. However, recommended and 75% of recommended rates of neem oil coated urea significantly improved the 1000 grain weight than recommended rate of uncoated urea.

**Harvest index %:** The physiological effectiveness of a crop to convert dry matter into economic yield is calculated by the harvest index formula. Harvest index is the ratio of economic yield over biological yield and multiplied by 100. Maximum harvest index (36.23% and 34.13%) was recorded in coated urea applied 75% to recommended dose of commercial urea in irrigated and barani wheat varieties.

The minimum harvest index of wheat (27.76% and 26.76%) in both varieties was recorded in control having no use of N fertilizer (Fig. 10). The outcome exposed that highest harvest index due to the coated urea fertilizer with 75% level for wheat.

**Grain yield per plant:** Figure 11 showed the influence of different rates of application of neem oil coated urea and uncoated urea on grain yield of wheat per plant. In both wheat varieties (Akbar-2019 and Barani 2017), neem oil coated urea yielded the maximum grain yield at 75% of the recommended rate of application, which was 4.3 and 4.2 g pot-1, while even its reduced application rates (50 and 25% of recommended rate) led to higher yields than control (2.5 and 2.2 g pot-1).

**Chemical parameters of soil and plant (pot)**

**Total nitrogen concentration in soil:** Nitrogen concentration in soil was also influenced greatly by the application of various levels of neem oil coated urea (Fig. 12). There was a maximum concentration of N in the soil (0.22 and 0.18% in both wheat varieties trials) when 75% neem oil coated urea of the recommended dose was applied. After 75% neem oil coated urea, uncoated urea induced second highest N concentration in soil by inducing 0.17 and 0.16% N with recommended rate of application. With regard to coating materials, neem oil applied at 75% of the recommended rate of application produced a higher level of nitrogen in soil than uncoated urea.

**Nitrogen concentration in root:** As given in Fig. 13 lowest N concentration in wheat roots was seen where no N fertilizer was applied, while N fertilizers in all levels and rates of application gave higher N concentration in wheat roots than control. Levels of coated urea at full, 75% of recommended rate gave higher N concentration in wheat roots than recommended rate of uncoated urea (0.88% and 0.83%) in both varieties. However, 50% of recommended rate induced slightly lower N in roots than recommended rate of uncoated urea.

**Nitrogen concentration in shoot:** Nitrogen concentration in shoot was found maximum in plots treated with 75% neem oil coated urea at recommended rate (0.70% in Akbar-2017) followed by uncoated urea at recommended rate (0.66%). Reduced rates of all levels of coated urea also gave excellent response; even 25% of recommended rate neem oil coated urea gave nitrogen concentration in shoot (0.42% and 0.36% respectively) best to negative control (Fig. 14). Furthermore, in barani wheat variety "Barani-2017" 75% neem oil coated urea gave more N concentration in shoot (0.62%) in comparison to all other treatments, as depicted in Fig. 14.

**Nitrogen concentration in grain:** The concentration of nitrogen (N) in the grains of wheat was highly variable among applied treatments, as given in Fig. 15. The lowest N concentration in the grains of wheat (0.39% in Akbar-2019 variety and 0.30% in Barani-2017 variety) was found where no N fertilizer was applied (control). While all N applied treatments at all rates significantly enhanced N concentration in grains of wheat in both varieties. Reduced rates of neem oil coated urea (75% of recommended rate) induced N concentrations in the grains of wheat varieties higher than that induced by uncoated urea at recommended rate.

## Discussion

In modern agriculture, chemical fertilizers are widely used (Yan *et al.*, 2010. It is important to pay special attention to fertilizers containing nitrogen (N), an essential macronutrient for plants (Li *et al.*, 2021). Numerous issues arise, however, when N fertilizer is introduced into the soil, due to the significant losses that occur during nitrification and subsequent denitrification processes (Belete *et al.*, 2018; Van-Eerd *et al.*, 2017). In several studies, applied N has been found to undergo several chemical processes as well as interact with soil bacteria and plant roots. (Castellano-Hinojosa *et al.*, 2019; Abbas *et al.*, 2020). In two successive steps, nitrifying bacteria transform ammonium into nitrite and then into nitrate. Nitrogen may be lost in the form of nitrous oxide or nitrite during this process (Zhu *et al.*, 2021). During nitrate leaching or denitrification processes, groundwater, surface water, and air pollution may occur as a result (Wang *et al.*, 2019). As a result, keeping an eye on the nitrogen dynamics of the soil is crucial. Using neem oil coated urea, a series of laboratory and pot trials have been conducted to boost agronomic yields.

In my first study, comparison for number of coating layers of neem oil on urea granules was investigated and different parameters were monitored like ammoinum and nitrate concentration. The release of fertilizer from a single coating was higher than from a double and triple coating at the early stages of fertilizer application, which may indicate that the coatings were inefficient. A triple coating significantly reduced the amount of release that might not meet plant requirements. The double layer coating, however, consistently released nitrogen at all intervals, meeting plant needs at all times. Due to the fact that coating thickness influences nutrient release, double coating layers might be a result of the thickness of the coating on the surface of urea granules. In a similar study Noor *et al.*, (2017) has also proved that double layer of coating showed more consistent release which was suitable to fulfill crop demand.

The results of pot experiment (Second study) were based on the performance of neem oil coated urea for improving the growth and yield of wheat crop. Compared to the control, all growth and physiological attributes, including plant height, chlorophyll content, fresh and dry weight of shoots, spike length, and spikelets per spike, were significantly higher with coated urea. Even reduced rate (75% of recommended) of coated urea also gave higher growth than uncoated urea. This might be due to the consistent supply of nitrogen from coated urea. Because of nitrogen plays vital role in the vegetative growth of plants. It also plays role in the physiological functioning of the plants. Ghafoor *et al.*, (2021) also proved that coated fertilizer enhanced the plant growth of wheat. The yield attributes of wheat like grains per spike, 1000 grains weight and grain yield per plant were also influenced highly with neem oil coated urea. However, highest yield was seen with the application neem oil coated urea at 75% of recommended rate. In line with our findings, Zhang *et al.*, (2020) described that nitrogen fertilization with consistent supply improved the growth of wheat. Several other strategies adopted to improve the nitrogen fertilization like deep placement and coated fertilizer application resulted in crop growth improvement in maize and rice (Wu *et al.*, 2017; Rychel *et al.*, 2020; Ghafoor *et al.*, 2021).

In wheat leaves, chlorophyll content was also influenced by all kinds of coated fertilizers. In comparison to uncoated urea, neem oil coated urea @ 75% induced higher chlorophyll contents. As nitrogen is a structural component of chlorophyll, its consistent supply during a crop plant's physiological growth could help increase its physiological activity. This might lead to higher chlorophyll contents at the physiological maturity of crop. In two similar experiments, Oad *et al.*, (2004) and Khalofah *et al.*, (2021) proved that coated N fertilizer and deep placement of N fertilizer lead to higher chlorophyll contents due to consistent N supply.

## Conclusions

Neem oil is an environmentally notorious material that can be used effectively to manage urea utilization efficiency issues based on the results of the experiment. It will also help to manage environmental problems associated with ammonia emission and nitrate leaching into groundwater due to fertilizer use efficiency. As a result, the farmer can increase his income and the environment suffers a minimum loss. It performed well under normal soil and environmental conditions when coated with urea. Among all other treatments, double-layer neem oil-coated urea (1%) showed the best results. Temperature and moisture levels increased, which resulted in an increase in ammonium and nitrate releases. Growth, yield, N concentration in different parts of the plant, and nitrogen use efficiency were the highest with neem oil coated urea 75% as compared with the recommended rate. Neem oil coated urea application is beneficial to agronimic parameters of wheat.

**Acknowledgments**

The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP2024R356), King Saud University, Riyadh, Saudi Arabia.

**References**

Abbas, Q., B. Yousaf, M.U. Ali, M.A.M. Munir, A. El-Naggar, J. Rinklebe and M. Naushad. 2020. Transformation pathways and fate of engineered nanoparticles (ENPs) in distinct interactive environmental compartments: A review. *Environ. Int.*, 138: 105646.

Ashraf, M.N., T. Aziz, M.A. Maqsood, H.M. Bilal, S. Raza, M. Zia, A. Mustafa, M. Xu and Y. Wang. 2019. Evaluating organic materials coating on urea as potential nitrification inhibitors for enhanced nitrogen recovery and growth of maize (*Zea mays*). *Int. J. Agric. Biol*., 22: 1102-1108.

Belete, F., N. Dechassa, A. Molla and T. Tana. 2018. Effect of nitrogen fertilizer rates on grain yield and nitrogen uptake and use efficiency of bread wheat (*Triticum aestivum* L.) varieties on the Vertisols of central highlands of Ethiopia. *Agri. Food Secur.*, 7: 1-12.

Byrne, M.P., J.T. Tobin, P.J. Forrestal, M. Danaher, C.G. Nkwonta, K. Richards, E. Cummins, S.A. Hogan and T.F. O'Callaghan. 2020. Urease and nitrification inhibitors As mitigation tools for greenhouse gas emissions in sustainable dairy systems: A review. *Sustainability,* 12: 6018.

Castellano-Hinojosa, A., J. González-López, A. Vallejo and E.J. Bedmar. 2019. Linking ammonia volatilization with moisture content and abundance of nitrification and denitrification genes in N-fertilized soils. *Microbial Probiotics for Agricultural Systems: Advances in Agronomic Use*, 29-43.

Fageria, N.K. 2014. Nitrogen harvest index and its association with crop yields. *J. Plant Nutr.*, 37: 795-810.

Fradgley, N.S., A.R. Bentley and S.M. Swarbreck. 2021. Defining the physiological determinants of low nitrogen requirement in wheat. *Biochem. Soc. Trans.*, 49: 609-616.

Frame, W. 2017. Ammonia volatilization from urea treated with NBPT and two nitrification inhibitors. *Agron. J.*, 109: 378-387.

Ghafoor, I., M. Habib-ur-Rahman, M. Ali, M. Afzal, W. Ahmed, T. Gaiser and A. Ghaffar. 2021. Slow-release nitrogen fertilizers enhance growth, yield, NUE in wheat crop and reduce nitrogen losses under an arid environment. *Environ. Sci. Pollut. Res.*, 28: 43528-43543.

Hansen, S., F.R. Berland, M. Stenberg, J. Stalenga, J.E. Olesen, M. Krauss, P. Radzikowski, J. Doltra, S. Nadeem, T. Torp and V. Pappa. 2019. Reviews and syntheses: Review of causes and sources of $N_2O$ emissions and $NO_3$ leaching from organic arable crop rotations. *Biogeosciences*, 16: 2795-2819.

Huddell, A.M., G.L. Galford, K.L. Tully, C. Crowley, C.A. Palm, C. Neill, J.E. Hickman and D.N. Menge. 2020. Meta-analysis on the potential for increasing nitrogen losses from intensifying tropical agriculture. *Global Change Biol.*, 26: 1668-1680.

Jackson, M.L. 1962. Soil chemical analysis: advanced course. UW-Madison Libraries Parallel Press, 1-854.

Joshi, A.G.J.K., J.K. Gupta, S.K. Choudhary and D.K. Paliwal. 2014. Efficiency of different nitrogen source, doses and split application on growth and yield of maize (*Zea mays* L.) in the Malwa region of Madhya Pradesh. *IOSR J. Agri. Veter. Sci*., 7(2): 2319-2372.

Keeney, D.R. and D.W. Nelson. 1989. Nitrogen inorganic forms. In*:* (Ed.): Page, A.L., *Methods of soil analysis*, part 2. Chemical and microbiological properties. Madison (WI): *Soil Science Society of America and American Society of Agronomy*, 643-698.

Khalofah, A., M.I. Khan, M. Arif, A. Hussain, R. Ullah, M. Irfan and R. Datta. 2021. Deep placement of nitrogen fertilizer improves yield, nitrogen use efficiency and economic returns of transplanted fine rice. *Plos One*, 16: e0247529.

Langholtz, M., B.H. Davison, H.I. Jager, L. Eaton, L.M. Baskaran, M. Davis and C.C. Brandt. 2021. Increased nitrogen use efficiency in crop production can provide economic and environmental benefits. *Sci. Total Environ.*, 758: 143602.

Li, H.F., S.D. An, L. Zhang, H. Peng, W. Ma, X. Meng and H.M. Ye. 2021. Urea fertilizer with precisely regulable slow-release performance by complexing with random copolyester. *J. Environ. Chem. Engin.*, 9(2): 105120.

Lindström, K. and S.A. Mousavi. 2020. Effectiveness of nitrogen fixation in rhizobia. *Microb. Biotechnol.*, 13(5):1314-1335.

Loick, N., E.R. Dixon, D. Abalos, A. Vallejo, G.P. Matthews, K.L. McGeough, R. Well, C.J. Watson, R.J. Laughlin, L.M. Cardenas. 2016. Denitrification as a source of nitric oxide emissions from incubated soil cores from a UK grassland soil. *Soil Biol. Biochem.*, 95: 1-7.

Lu, C. and H. Tian. 2017. Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: Shifted hot spots and nutrient imbalance. *Earth Sys. Sci. Data*, 9(1): 181-192.

Martinez-Feria, R.A., M.J. Castellano, R.N. Dietzel, M.J. Helmers, M. Liebman, I. Huber and S.V. Archontoulis. 2018. Linking crop-and soil-based approaches to evaluate system nitrogen-use efficiency and tradeoffs. *Agri. Ecosys. Environ.*, 256: 131-143.

Meena, A.K., D.K. Singh, P.C. Pandey and G. Nanda. 2018. Growth, yield, economics, and nitrogen use efficiency of transplanted rice (*Oryza sativa* L.) as influenced by different nitrogen management practices through neem (*Azadirachta indica*) coated urea. *Int. J. Chem. Stud.*, 6: 1388-1395.

Noor, S., M. Yaseen, M. Naveed and R. Ahmad. 2017. Use of controlled release phosphatic fertilizer to improve growth, yield and phosphorus use efficiency of wheat crop. *Pak. J. Agri. Sci.*, 54: 541-547.

Oad, F.C., U.A. Buriro and S.K. Agha. 2004. Effect of organic and inorganic fertilizer application on maize fodder production. *Asian J. Plant Sci.*, 3: 375-377.

Ogle, S.M., L. Olander, L. Wollenberg, T. Rosenstock, F. Tubiello, K. Paustian, L. Buendia, A. Nihart and P. Smith. 2014. Reducing greenhouse gas emissions and adapting agricultural management for climate change in developing countries: Providing the basis for action. *Global Change Biol.*, 20: 1-6.

Pfromm, P.H. 2017. Towards sustainable agriculture: Fossil-free ammonia. *J. Renew. Sustain. Energy*., 9: 034702.

Rychel, K. K.H. Meurer, G. Börjesson, M. Strömgren, G.T. Getahun, H. Kirchmann and T. Kätterer. 2020. Deep N fertilizer placement mitigated $N_2O$ emissions in a Swedish field trial with cereals. *Nutr. Cycl. Agroecosys.*, 118: 133-148.

Sha, Z., X. Ma, J. Wang, T. Lv, Q. Li, T. Misselbrook and X. Liu. 2020. Effect of N stabilizers on fertilizer-N fate in the soil-crop system: A meta-analysis. *Agri. Ecosys. Environ.*, 290: 106763.

Shaviv, A. and R.L. Mikkelsen. 1993. Controlled-release fertilizers to increase efficiency of nutrient use and minimize environmental degradation-A review. *Fert. Res.*, 35: 1-12.

Shewry, P.R. 2009. Wheat. Darwin review. *J. Exp. Bot*., 60: 1537-1553.

Singh, A., A. Jaswal and M. Singh. 2019. Impact of neem coated urea on rice yield and nutrient use efficiency (NUE). *Agri. Rev.*, 40: 70-74.

Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and Procedures of Statistics, 2nd edition. McGraw Hill Inc., New York, USA.

Suter, H., S.K. Lam, C. Walker and D. Chen. 2020. Enhanced efficiency fertilizers reduce nitrous oxide emissions and improve fertiliser 15N recovery in a Southern Australian pasture. *Sci. Total Environ.*, 699: 134147.

Thind, H.S., R.P.S. Pannu, R.K. Gupta, M. Vashistha, J. Singh and A. Kumar. 2010. Relative performance of neem (*Azadirachta indica*) coated urea vis-a-vis ordinary urea applied to rice on the basis of soil test or following need based nitrogen management using leaf colour chart. *Nutr. Cycl. Agroecosys.*, 87: 1-8.

Umar, W., M.A. Ayub, M.Z.U. Rehman, H.R Ahmad, Z.U.R. Farooqi, A. Shahzad, U. Rehman, A. Mustafa and M. Nadeem. 2020. Nitrogen and phosphorus use efficiency in agroecosystems. *Resour. Use Efficien. Agri.*, 213-257.

Valkama, E., K. Rankinen, P. Virkajärvi, T. Salo, P. Kapuinen and E. Turtola. 2016. Nitrogen fertilization of grass leys: Yield production and risk of N leaching. *Agri. Ecosys. Environ.*, 230: 341-352.

Van-Eerd, L.L., J.J.D. Turnbull, C.J. Bakker, R.J. Vyn, A.W McKeown and S.M. Westerveld. 2017. Comparing soluble to controlled-release nitrogen fertilizers: Storage cabbage yield, profit margins, and N use efficiency. *Can. J. Plant Sci.*, 98: 815-829.

Vitousek, P.M., R. Naylor, T. Crews, M. David, L. Drinkwater, E. Holland and F.S. Zhang. 2009. Nutrient imbalances in agricultural development. *Science*, 324(5934): 1519-1520.

Wang, S., X. Zhao, G. Xing, Y. Yang, M. Zhang and H. Chen. 2015. Improving grain yield and reducing N loss using polymer-coated urea in southeast China. *Agron. Sustain. Develop.*, 35: 1103-1115.

Wang, Y., H. Ying, Y. Yin, H. Zheng and Z. Cui. 2019. Estimating soil nitrate leaching of nitrogen fertilizer from global meta-analysis. *Sci. Total Environ.*, 657: 96-102.

Wolf, B. 1982. A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. *Comm. Soil Sci. Plant Anal.*, 13: 1035-1059.

Wu, D., M. Senbayram, R. Well, N. Brüggemann, B. Pfeiffer, N. Loick and R. Bol. 2017. Nitrification inhibitors mitigate N2O emissions more effectively under straw-induced conditions favoring denitrification. *Soil Biol. Biochem.*, 104: 197-207.

Xie, Y., L. Tang, L. Yang, Y. Zhang, H. Song, C. Tian, X. Rong and Y. Han. 2020. Polymer-coated urea effects on maize yield and nitrogen losses for hilly land of southern China. *Nutr. Cycl. Agroecosys.*, 116: 299-312.

Yan, X. and W. Gong. 2010. The role of chemical and organic fertilizers on yield, yield variability and carbon sequestration—results of a 19-year experiment. *Plant and Soil*, 331: 471-480.

Ying, H., Y. Yin, H. Zheng, Y. Wang, Q. Zhang, Y. Xue, D. Stefanovski, Z. Cui and Z. Dou. 2019. Newer and select maize, wheat, and rice varieties can help mitigate N footprint while producing more grain. *Global Change Biol.*, 25: 4273-4281.

Zaman, M., S. Zaman, M.L. Nguyen, T.J. Smith and S. Nawaz. 2013. The effect of urease and nitrification inhibitors on ammonia and nitrous oxide emissions from simulated urine patches in pastoral system: A two-year study. *Sci. Total Environ.*, 465: 97-106.

Zhang, L. Z. Liang, Y. Hu, U. Schmidhalter, W. Zhang, S. Ruan and X. Chen. 2021. Integrated assessment of agronomic, environmental and ecosystem economic benefits of blending use of controlled-release and common urea in wheat production. *J. Cleaner Prod.*, 287: 125572.

Zhang, L., X. He, Z. Liang, W. Zhang, C. Zou and X. Chen. 2020. Tiller development affected by nitrogen fertilization in a high-yielding wheat production system. *Crop Sci.*, 60: 1034-1047.

Zhang, W., Z. Liang, X. He, Wang, X. Shi, C. Zou and X. Chen. 2019. The effects of controlled release urea on maize productivity and reactive nitrogen losses: A meta-analysis. *Environ. Pollut.*, 246: 559-565.

Zhu, X., J. Shen, Y. Li, X. Liu, W. Xu, F. Zhou and J. Wu. 2021. Nitrogen emission and deposition budget in an agricultural catchment in subtropical central China. *Environ. Pollut.*, 289: 117870.

(Received for publication 10 January 2024)