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IMPACT OF SLOW-RELEASE NITROGEN FERTILIZER ON NITRATE LEACHING, NITROGEN DISTRIBUTION, AND GRAIN YIELD OF WHEAT (*TRITICUM AESTIVUM* L.)

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SUMMARY

An executed laboratory study gauged the effect of slow-release fertilizers on nitrate leaching and its distribution in the soil profile and yield of wheat (*Triticum aestivum* L.). A comparison between numbers of coating layers (1, 2, and 3 layers) of neem oil on urea granules caused an investigation, with different parameters monitored. Results revealed a higher nitrogen (N) release arose from a single coating than the double and triple coatings at the early stage of fertilizer application, indicating a poor performance and less efficiency. Triple coating effectively suppressed the release, which could unmatched plant needs. Afterward, a second study continued in pots to compare the efficiency of different levels (25%, 50%, and 75%) of neem oil-coated fertilizer with untreated fertilizer and the control on wheat's physiological parameters and yield. It was noteworthy that a reduced dose (75%) of fertilizer gave a better response for improving growth parameters and nutritional status of wheat. Overall, urea fertilizer coated with neem oil improved crop management and reduced nitrogen loss in environmental consequences, which usually resulted from adding traditional fertilizers. Thus, using naturally occurring neem oil as coating material could improve wheat yield and reduce nitrogen losses.

Keywords: Wheat (*T. aestivum* L.), nitrogen, neem oil-coated urea, slow-release fertilizer, grain yield

Key findings: Utilization of suitable coating material can slow down the release of N from urea. This study proved the use of neem oil as a coating material significantly reduced the N release and N losses from urea fertilizer used in the wheat crop.

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INTRODUCTION

The use of synthetic fertilizers for cultivation improved crop yield, but reports on several negative impacts have also gone out on many environmental factors, such as, greenhouse gas emissions, water quality, and climate effects (Savci, 2012). The N fertilizer is a commonly used stimulant to boost arable crop productivity (Martinez-Feria *et al*., 2018). When N supply exceeds plant requirements, nitrate nitrogen NO_3-N leaching occurs (Hansen *et al*., 2019; Umar *et al*., 2020), causing ammonia NH_3 volatilization (Ying *et al*., 2019), and $N_2O/NO/N_2$ emission (Loick *et al*., 2016; Huddell *et al*., 2020). These result in non-point-source pollution (Sha *et al*., 2020), global warming (Ogle *et al*., 2014), and other adverse environmental influences. Among these losses, the major N loss is via ammonia volatilization (Frame, 2017; Ashraf *et al*., 2019). Therefore, it is crucial to adopt some strategies to reduce negative environmental impacts, while maintaining crop yields (Suter *et al*., 2020).

Wheat (*Triticum aestivum* L.) is the most commonly grown and consumed crop in temperate regions. Whole grain products contain vitamins, minerals, amino acids, fiber, and phytochemicals essential for nutrition (Shewry, 2009). Nitrogen is crucial for wheat production, which feeds millions of people. One of the main nutrients limiting wheat yield is nitrogen (Fradgley *et al*., 2021). Wheat varieties also use N differently to increase yield (Belete *et al*., 2018). Thus, applying the correct rate of nitrogen fertilizer is considerably the main cause of improving wheat grain yield, enhancing N uptake and nutrient use efficiency (NUE). However, N losses due to volatilization lead to reduced yield and NUE of wheat. Recent years revealed numerous opportunities to have improved NUE and reduced N losses (Langholtz *et al*., 2021). For example, urease and nitrification inhibitors lessen N losses (Byrne *et al*., 2020); however, ordinary farmers cannot afford urease and nitrification inhibitors (Zaman *et al*., 2013). Nitrogen fixation improvement can proceed through breeding, although, it takes time.

Therefore, the use of controlled release urea (CRU) is a recommendation to moderate environmental damage caused by urea application. At a soil pH of 6.0, CRU effectively reduces N_2O emissions and NH_3 volatilization (Zhang *et al*., 2019). Mixing reduced ammonia volatilization caused losses by 17%–20% compared with the uncoated urea (Zhang *et al*., 2021). Another cheaper and more effective method to reduce nitrogen losses and increase NUE and crop yield is to encapsulate urea with polymers (Wang *et al*., 2015; Xie *et al*., 2020). Similarly, nitrogen loss can also diminish by applying slow-release fertilizers (Joshi *et al.,* 2014).

In some countries, a traditional method of mixing urea with neem cake to increase nitrogen utilization efficiency is prevalent (Singh *et al*., 2019). Thus, urea coating with neem oil is applicable to reduce N losses, as the slow release of N transpires in plants throughout their life cycle (Thind *et al*., 2010). Neem products, when used along with urea, can increase NUE in crops (Meena *et al.,* 2018). Therefore, the planned investigations sought to analyze the efficiency of different coating layers and levels of neem oil-coated urea for improving yield of wheat at two different locations (irrigated and non-irrigated), in comparison with the commercial urea.

MATERIALS AND METHODS

Experimental locations and treatments

A laboratory and pot experiment commenced at the Department of Soil and Environmental Sciences, College of Agriculture, University of Sargodha, Sargodha (UOS), to optimize the use of neem oil for coating on urea. It aimed at enhancing the nitrogen use efficiency of wheat (*T. aestivum* L.) crop.

Soil analysis

The soil used in the laboratory and pot experiments bore bulk sampling from the top 15-cm layer. The composite sample reached pre-analysis for various physiochemical parameters at the departmental laboratory.

The details of the tested soil sample are available in Table 1. The first study conducted at the laboratory was in a climate growth chamber under specific temperatures (temperature ranged from 15 °C to 25 °C), relative humidity (50%), and lighting (dark period by a light period of 2 h, followed by 4–12 h of darkness in a 24 h cycle, 500 μmoles/m^2/s) conditions to check the effect of different number of coatings of neem oil (1.0%) on urea fertilizer for N release. The experimental design had a completely randomized design (CRD), with each treatment replicated three times. The treatment plan consisted of T1 as uncoated urea (control), T2 as single-coated urea, T3 as double-coated urea, and T4 as triple-coated urea with neem oil. Four treatment sets ensued for determining $NH_4^{1+}$and NO_3^- release at 10, 20, and 30-day intervals, along with three replications.

Afterward, the conduct of a pot experiment followed in the wire house of the Department of Soil and Environmental Sciences, UOS, to evaluate the effect of neem oil-coated urea rates on wheat growth and yield. Selecting the best number of coating layers (double) resulted from the laboratory experiment. The treatment plan comprised T1 as the control (without N fertilizer); T2 as uncoated urea; T3 as coated urea at 75% of recommended rate of neem oil coating; T4 as coated urea at 50% of recommended rate of neem oil coating, and T5 as coated urea at 25% of recommended rate of neem oil coating. Two wheat varieties, i.e., rainfed (Barani-2017) and Irrigated (Akbar-2019) were tested samples at two different locations.

Pots filled with pre-analyzed soil were at 10 kg per pot. Pots arrangement was according to the CRD for five treatments with three replications. Applying tap water to all pots to achieve field capacity level used a retention curve method. At field capacity, the sowing of five seeds of both wheat varieties "Barani-2017 and Akbar-2019" ensued in respective pots. After germination, two plants remained in each pot by manual thinning. Urea fertilizer served as the N source, while applying SSP and SOP as P and K sources had the rate of 120: 90: 60 kg ha^{-1} (N: P: K). All P and K application occurred at sowing, while urea (in coated and uncoated form) treatment happened with first irrigation. Applying uncoated urea was at a recommended rate, while coated fertilizers' treatment employed 75%, 50%, and 25% of the recommended rates of N. All other agronomic practices for pest control and growth requirements were operational, as and when needed.

Measurement of ammonium, nitrate, and cumulative nitrogen

A different number of coatings of neem oil on urea fertilizer was successful in the laboratory. Neem oil coating included single, double, and triple layers of urea. Field capacity

Table 1. Physicochemical properties of soil taken for the experiments.

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

maintenance on each after 24 h, with NH_4^+ and NO_3^- measured after 10, 20, and 30 days of incubation through the indophenol blue and phenol disulphonic acid methods, respectively (Keeney and Nelson, 1989). Calculation for cumulative N followed the sum of ammonium and nitrate concentration.

Evaluation of plant growth and yield of wheat

At the crop's physiological maturity, plant height measurement used a meter rod. After harvesting of wheat crops, noting yield and shoot dry weight of wheat ensued.

Chemical analysis of soil and plant

In all studies, the gathered soil samples from all treatments and many soil parameters incurred measuring. The determination of pH and EC values of a 1/5 (w/v) water-based soil extract transpired, using a pH meter to measure the pH value (Crison mod.2001, Barcelona, Spain), with the EC measured engaging a conductivity meter (Crison micro CM2200, Barcelona, Spain). Total nitrogen (N), total carbon (C), and soil organic carbon (SOC) measuring utilized a combustion gas chromatography in a Thermo Finnigan (Franklin, MA, USA) elemental analyzer called Flash EA 1112. Nitrogen content in grain, straw, and roots underwent chemical measurement in the departmental laboratory. The study followed the procedure suggested by Wolf (1982) to digest grain, straw, and root samples with sulfuric acid and hydrogen peroxide, with the Kjeldahl method determining nitrogen concentration in digested plant samples (Jackson, 2005).

Statistical analysis

Data collected from lab and pot experiments sustained analysis using the completely randomized design (CRD). All analysis on the collected data used the Statistics 8.1 software, and conducting mean comparison followed the least significant difference ($LSD_{0.05}$) test for homogeneous groups (Steel *et al*., 1997).

RESULTS

NH_4^+ concentration in soil with intervals (10, 20, 30 days)

Ammonium release from uncoated urea was the maximum at the first interval. But, its enormous reduction occurred at latter intervals. Meanwhile, all coating layers with neem oil (single, double, triple) lowered the ammonium release at the first interval than the uncoated urea; however, a significant rise showed at the second interval. At the third interval, though ammonium release was significantly lower than the second interval, it was still higher than the discharge at the first interval. Although, the maximum ammonium release came from the double coating of neem oil at 1,598 mg kg^{-1}. Among the number of coating layers, double coating released the highest ammonium at second and third intervals for all coating layers (Table 2).

Table 2. Impact of coating layers on NH_4^+, NO_3^- and cumulative N concentrations in the soil at 10, 20, and 30 days intervals.

Fertilizer sources	Number of coating layers	NH_4^+ (mg kg^{-1})			NO_3^- (mg kg^{-1})			Cumulative N (mg kg^{-1})		
		Time interval (days)			Time interval (days)			Time interval (days)		
		10	20	30	10	20	30	10	20	30
Negative control	No coating	1.7 k	0.7 k	0.4 k	1.0 h	0.6 h	0.5 h	2.7 j	0.13 j	0.9 j
Positive control	Uncoated	1464 c	432 h	241 j	1176 a	851 b	315 ef	2640 a	1283 f	556 i
Neem oil-coated urea	Single	465 g	1510 b	969 de	279 f	438 e	671 c	744 g	1948 bc	1640 cd
	Double	461 g	1598 a	974 ef	273 f	597 d	684 c	734 g	2195 b	1658 c
	Triple	355 i	1189 d	893 f	256 g	431 e	614 cd	611 h	1620 d	1507 e

NO_3^- concentration in soil with intervals (10, 20, 30 days)

The nitrate release was significantly lower in coated treatments than the uncoated treatment at the first and second intervals. At the third interval, nitrate concentration from coated treatments indicated a little increase versus the uncoated treatment (Table 2). At the first interval, the maximum nitrate concentration (1,176.0 mg kg^{-1}) was evident in the soil treated with uncoated urea, while the minimum was with the negative control (1.0 mg kg^{-1}). The single coating released more nitrate in the soil than the double and triple coating for neem oil at the first interval. But, at the third interval, a double layer coating of neem oil gave a higher nitrate release than the single and triple layer coatings.

Cumulative N concentration in soil with intervals (10, 20, 30 days)

Cumulative nitrogen showed a consistent decline in the negative control and uncoated urea treatments. Meanwhile, in coated fertilizers, cumulative nitrogen concentration provided a rise in the first and second intervals, then slightly lowered at the third interval with the value still higher than that of the first interval (Table 2). At the first interval, maximum cumulative N release was notable for the uncoated urea (2,640.0 mg kg^{-1}), while among coated treatments, the double coating of neem oil induced the highest cumulative N release at 734 mg kg^{-1}. At the second interval, a maximum release of cumulative N resulted from the double coating of neem oil (1.0%). It was noticeable that cumulative N release was more consistent from the double layer coating of all number of coating layers. The highest cumulative N release (2,195.0 mg kg^{-1}) came from the double coating of neem oil at 20 days interval.

Agronomic performance of wheat

Plant height

Maximum plant height (84.67 cm) resulted with the 75% neem coated urea compared with the recommended rate of commercial urea applied, followed by the uncoated recommended urea (77.67 cm) in irrigated wheat variety Akbar-2019. Reduced rates of all types of coated urea also gave excellent response; even 25% of the recommended rate of neem oil coated urea gave a plant height (70.33 cm) superior to the negative control (Figure 1). Furthermore, in the rainfed wheat variety Barani-2017, the 75% neem oil coated urea gave a taller plant height (79.3 cm) than all other treatments.

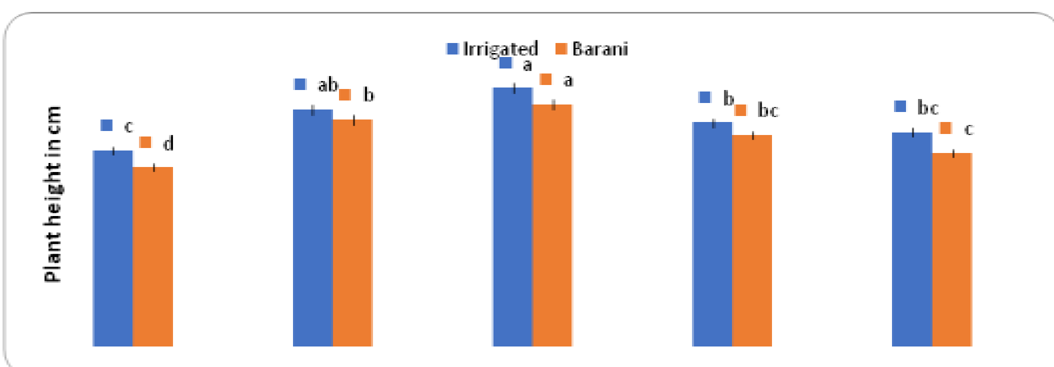

Figure 1. Impact of different rates of neem oil-coated urea on plant height (cm) of wheat.

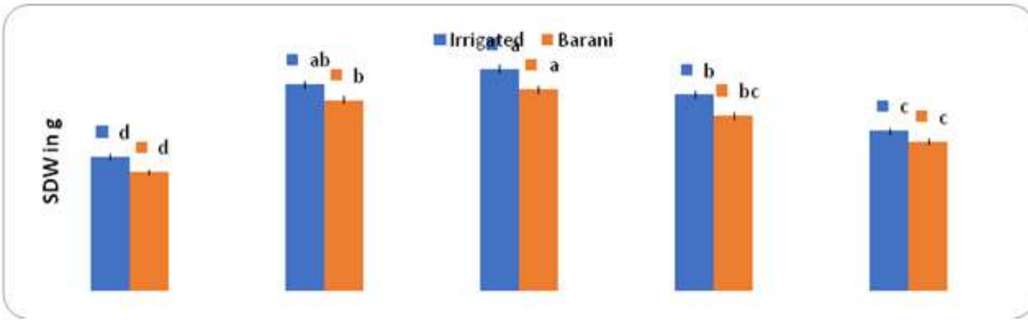

Figure 2. Impact of different rates of neem oil-coated urea on shoot dry weight (g/plant) of wheat.

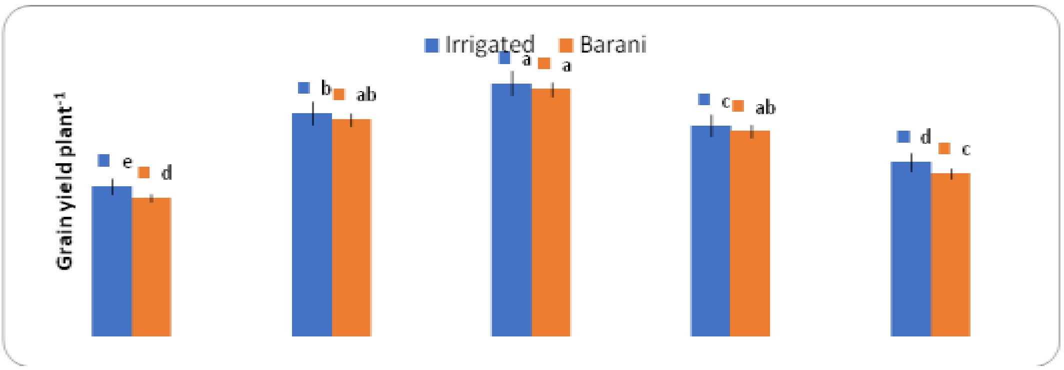

Figure 3. Impact of different rates of neem oil-coated urea on grain yield (g) of wheat.

Dry weight of shoot

Shoot dry weight (SDW) elevated in the plant wheat treated with all levels of coated urea (Figure 2). Even reduced rates (75%, 50%, and 25% of recommended rate) of all coated urea fertilizer induced higher dry biomass of wheat than the recommended rate of uncoated urea, indicating a net saving of urea fertilizer up to 25% to 50%. However, the maximum shoot dry weight was evident in the treatment of neem oil coated urea at 75% (0.43 and 0.39 g in both wheat varieties), followed by the recommended rate of uncoated urea (0.40 and 0.37 g). The control treatment induced shoot dry weights up to 0.26 and 0.23 g, which were lower than all other treatments.

Grain yield per pot

Figure 3 showed the influence of different rates of application of neem oil-coated urea and uncoated urea on grain yield of wheat per plant. Maximum grain yield emerged with the neem oil-coated urea at 75% of the recommended rate of application at 4.2 and 4.1 g pot^{-1} in both wheat varieties (Akbar-2019 and Barani-2017). Even its reduced rates (50% and 25% of recommended rates) gave more grain yield than the control (2.5 and 2.3 g pot^{-1}, respectively).

Nitrogen concentration in soil and plant

Total nitrogen concentration in soil

The N concentration in soil gained great influences from the application of various levels of neem oil-coated urea (Table 3). The maximum N concentrations in soil (0.21% and 0.19% in both wheat varieties' trial) were noteworthy with the 75% neem oil-coated urea recommended dose application. After the 75% neem oil-coated urea, uncoated urea induced

Table 3. Impact of different rates of neem oil-coated urea on N content of soil and plant (Total N % in soil, root, shoot, and grain).

Treatments	Soil N (%)		Root N (%)		Shoot N (%)		Grain N (%)	
	Akbar-2019	Barani-2017	Akbar-2019	Barani-2017	Akbar-2019	Barani-2017	Akbar-2019	Barani-2017
Control	0.15 c	0.14 d	0.38 c	0.35 c	0.27 e	0.19 d	0.38 d	0.27 d
Uncoated urea	0.18 ab	0.17 ab	0.76 ab	0.69 ab	0.64 b	0.61 ab	0.81 b	0.75 ab
Coated urea 75%	0.21 a	0.19 a	0.87 a	0.82 a	0.71 a	0.63 a	0.98 a	0.87 a
Coated urea 50%	0.17 b	0.17 ab	0.74 b	0.66 b	0.59 c	0.52 c	0.73 c	0.67 b
Coated urea 25%	0.17 b	0.16 c	0.59 bc	0.51 bc	0.41 d	0.34 d	0.56 cd	0.41 cd

the second-highest N concentration in soil by inducing 0.18% and 0.17% N with a recommended rate of application. Among coating materials, neem oil 75% rate showed more efficiency by causing more N concentration in the soil than the uncoated urea at the recommended rate of application.

Nitrogen concentration in root

The lowest N concentration in wheat roots manifested with the non-N fertilizer treatment, while N fertilizers in all levels and rates of application gave higher N concentrations in wheat roots than the control (Table 3). Levels of coated urea at full, 75% of recommended rate gave the maximum N concentration in wheat roots versus the recommended rate of uncoated urea (0.87% and 0.82%) in both varieties. However, 50% of the recommended rate induced a slightly lower N in roots than the recommended rate of uncoated urea.

Nitrogen concentration in shoot

Nitrogen concentration in shoot appeared maximum in plots treated with 75% neem oil-coated urea at the recommended rate (0.71% in Akbar-2017), followed by the uncoated urea at a recommended rate (0.64%). Reduced rates of all levels of coated urea also gave excellent response; even 25% of the recommended rate neem oil-coated urea gave high nitrogen concentrations in shoot (0.41% and 0.34%, respectively), better than the negative control. Furthermore, in rainfed wheat variety Barani-2017, 75% neem oil-coated urea gave more N concentration in shoots (0.63%) than all other treatments (Table 3).

Nitrogen concentration in grain

The concentration of nitrogen (N) in the grains of wheat was highly variable among applied treatments, as given in Table 3. The lowest N concentration in the grains of wheat (0.38% in Akbar-2019 and 0.27% in Barani-2017) occurred with the no N fertilizer (control). Meanwhile, all N applied treatments at all rates significantly enhanced the 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 with the uncoated urea at recommended rates.

DISCUSSION

Chemical fertilizers are a widely used treatment in modern agriculture. Fertilizers containing nitrogen (N), a critical macronutrient for all plants, are of special attention. Numerous issues revealed an association with the application of chemical fertilizers due to the significant losses occurring during nitrification and subsequent denitrification processes. The use of coated urea can help mitigate such losses. Neem oil can slow down the activities of bacteria (nitrosomonas and nitrobacter) responsible for nitrification, and thus, lessening N losses

(Belete *et al*., 2018). The results of these studies also confirmed such findings. In first studies, the comparison for the number of coating layers of neem oil on urea granules reached scrutiny, with different parameters monitored. Comparing the number of coating layers, a higher release was visible from the single coating than the double and triple coating at the early stage of fertilizer application. The triple coating extraordinarily suppressed the release, which could not match plant needs. However, the double-layer coating released nitrogen consistently at all intervals, enhancing plant requirements efficiently. As coating thickness controls the release of nutrients, therefore, these results in double-coating layers might be the consequence of coating thickness on the surface of urea granule. In a similar study, Noor *et al*. (2017) has proven the double layer of coating showed more consistent releases, suitable to fulfill crop demands.

The results of the pot experiment (second study) relied on the performance of neem oil-coated urea for improving the growth and yield of wheat crops. The results revealed all growth and physiological attributes, i.e., plant height and dry weight of shoots, appeared significantly higher with all levels of coated urea than the control. Even a reduced rate (75% of the recommended) of coated urea gave a higher growth than the uncoated urea. This might be due to the consistent supply of nitrogen from coated urea because nitrogen plays a vital role in the vegetative growth of plants. It is also crucial in the physiological functioning of the plants. Ghafoor *et al*. (2021) also proved the coated fertilizer enhanced plant growth of the wheat. The yield attributes of wheat, such as, grain yield per plant acquired great influences from the neem oil-coated urea. However, the highest yield emerged with the application of neem oil-coated urea at the 75% of recommended rate. Similar to our findings, Zhang *et al*. (2021) described the nitrogen fertilization with consistent supply improved wheat growth. Several other strategies adopted to improve the nitrogen fertilization, such as, deep placement and coated fertilizer application resulted in crop growth enhancement in maize and rice (Ghafoor *et al*., 2021). The N concentration in soil and plant parts was also maximum in the treatment of 75% reduced rate of neem oil-coated urea. Likewise, coating plants with neem oil increases grain production, with N concentration in wheat grains across all soil types examined (Singh *et al*., 2019).

Numerous studies have shown applying N undergoes several mechanisms through chemical processes and interaction with soil bacteria, as well as, the roots of plants (Abbas *et al*., 2020). Nitrifying bacteria first convert ammonium to nitrite, and then, to nitrate in two successive processes. Nitrogen may be lost in the form of nitrous oxide or nitrite during this process. It is possible that nitrate leaching or denitrification processes may potentially result in groundwater, surface water, and air pollution occurring from this reaction (Wang *et al*., 2019; Alaamer *et al*., 2024; Naas and Al-Majidi, 2024). Thus, it is vital to keep an eye on the soil's nitrogen dynamics. A series of laboratory and pot experiments progressed to enhance the growth and yield of wheat by applying neem oil-coated urea.

CONCLUSIONS

Neem oil-coated urea fertilizer facilitates crop management and reduces the environmental impact generated by nitrogen losses, usually outputs of traditional fertilizers. Thus, using naturally occurring neem oil as a coating material had the potential to enhance the growth, yield, and nitrogen use efficiencies in wheat (*T. aestivum* L.) crops and control negative effects on the environment.

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