

EVALUATION OF POLYMER-COATED UREA TO REDUCE NITROGEN LOSSES IN SOIL AND ITS APPLICATION TO OKRA *ABELMOSCHUS ESCULENTUS* (L.) MOENCH

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Abstract *The application of fertilizer aims to maximize crop yields and provide essential nutrients to plants. Efficiency problems, decreased yields, and environmental impact can result from nitrogen loss by volatilization and leaching. The delayed release of nitrogen can be achieved by coating urea with biodegradable compounds. The influence of several polymer-coated urea formulations and standard urea on nitrogen availability to okra plants was investigated in this study using lab and pot assessments. Key parameters were determined using soil samples taken at a depth of 15 cm. In the lab incubation, control, conventional urea, starch polymer-coated urea, neem oil-coated urea, and acacia gum-coated urea treatments were examined over 35 days. Soil samples were collected and examined on particular days. Okra plants were grown up to the flag leaf stage in the pot experiment using the same treatments. Data analysis demonstrated higher ammonium-nitrate and nitrate nitrogen concentrations in soils treated with polymer-coated urea, with acacia gum-coated urea exhibiting the slowest release. As release rates varied over time, nitrogen levels increased. Polymer-coated urea reduced ammonium loss, increased nutrient absorption, and boosted plant growth when compared to conventional urea. Urea with acacia gum coating functioned remarkably well, leading to increased nitrogen absorption. In conclusion, biodegradable polymer-coated urea increases nitrogen usage efficiency, decreasing losses and enhancing plant development, particularly when combined with acacia gum-coated urea. The results have practical significance for agricultural sustainability and the preservation of the environment.*

Keywords: *Nitrogen loss; Okra; Polymer coated urea; Biodegradable; Incubation*

Introduction

Okra, a popular vegetable with nutritional and medicinal benefits, rapidly perishes postharvest due to changes in weight, softness, color, and damage sensitivity. These factors affect the fruit's quality, storage suitability, marketability, and transportation, despite numerous studies on postharvest practices (Aloui et al. 2017; Al-Naamani et al. 2018; Phornvillay et al. 2019; Sun et al. 2021; Dong et al. 2023; Li et al. 2023) Improving postharvest practices and enhancing nitrogen use efficiency is crucial for maintaining okra quality and reducing agricultural and environmental impacts. The large-scale use of nitrogen fertilizers contributes to atmospheric concentrations of greenhouse gases and air pollutants like nitrous oxide and ammonia (Tian et al. 2015). Studying the impact of applied nitrogen on the

agroecological system can lead to increased nitrogen use efficiency, sustainable agriculture, and economic gains (Langholtz et al. 2021). Traditional urea usage inefficiency leads to excessive nitrogen loss in agriculture, posing various risks and environmental contamination (Amberger 2006). Nitrogen competition from mineral fertilizers poses a significant issue, as plants only absorb and use 20%-30% of available nitrogen, causing environmental harm and subsurface water damage. Nitrogen is crucial for plant metabolism, and synthesis of chlorophyll, proteins, and phytohormones, and is not replaced by alternative nutrients (Trenkel 1997). Polymer-coated urea fertilizers aim to reduce environmental losses and increase usage efficiency by aligning nitrogen supply with crop demand,

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addressing concerns about nitrogen availability and environmental impacts in fertilizer administration (Grant et al. 2012).

Urea is the primary nitrogen fertilizer, accounting for over 50% of global agricultural nitrogen input (Lasisi et al. 2020). Urea is a diamide of carbonic acid (NH₂)₂CO, containing 46% nitrogen (Gould et al. 1986; Galloway et al. 2008; Coskun et al. 2017). Rapid ammonification occurs in soil due to the hydrolysis of the soil enzyme urease, secreted by microorganisms. This process generates gaseous ammonia and CO₂, which evaporates in agricultural soils. Ammonium carbonate is produced when urea partially dissolves in soil water. Nitrification, facilitated by soil microorganisms, oxidizes NH₄⁺ to NO₃⁻. Some ammonium is immobilized by organisms and stabilized by soil organs. The resulting nitrites and nitrates are used as plant fertilizers. However, nitrates can leach into groundwater, eutrophication water resources. Denitrifying soil bacteria convert nitrates into nitrogen-containing gases, and nitrogen loss from fertilizers may occur (Korsakov et al. 2023).

Coated slow-release fertilizer is an innovative product that adjusts the nutrient-release characteristics of standard fertilizer by adding supplementary ingredients, aiming to lower costs by adjusting nitrogen delivery to individual crop needs (Harvey et al. 2016; Li et al. 2021). Hydrophobic inorganic or organic coating materials are frequently employed as constraints. A variety of hydrophobic inorganic and organic coating materials, as well as combinations, have been used to improve slow-release fertilizers. One of the most researched inorganic compounds is sulfur (Li et al. 2022). Organic polymers are the primary sustained-release and controlled-release coating materials, but semisynthetic polymers are also being explored (Tan et al. 2021) is the most common, and it is a novel polymer material created by the grafting or copolymerization of many types of naturally occurring and synthetically modified polymers (Kwon et al. 2021; Haroon et al., 2024; Hunajid and Gokce, 2024; Sami et al., 2023; Mushtaq et al., 2024). Superabsorbent resin (SAR) is a novel functional polymer material with a three-dimensional network structure, strong hydrophilic groups, and crosslinking (Cheng et al. 2019). Qiao et al. described a method for creating water-retentive, slow-release urea fertilizers by treating fertilizer surfaces with SAR, resulting in a double coating. Potato starch-coated fertilizer delivers minimal nitrogen into the soil, taking 100 hours to fully release. Charge and connecting bridge elements regulate fertilizer discharge in a resin-based gel network (Qiao et al. 2016). Slow-release coated

fertilizers can be applied to the soil to increase yield and reduce nitrogen loss from leaching (Abbas et al., 2024ab; Arshad et al., 2024; Rehman et al., 2024; Liu et al. 2013). Synthetic active ingredients (SAR) derived from renewable, biodegradable, and environmentally friendly natural resources like cellulose alginate and inorganic clay minerals are gaining widespread acceptance (Khalid et al. 2018; Chaudhuri et al. 2022; Thien et al. 2022). The research aims to create optimal urea coatings for slow-release urea production, evaluate their soil nutrient release, and test the best slow-release fertilizer in a pot experiment with okra for growth and absorption.

Materials and methods

Formulation of coated urea

Three coating materials, acacia gum, neem oil, and starch, were selected based on cost-effectiveness and accessibility. These materials were used to prepare three controlled-release urea fertilizers (CRFs) by applying a 10% w/w solution through spraying. The resulting fertilizers were subjected to an 8-hour drying and stored in air-tight jars for further assessment (Figure 1).

To assess the nitrogen release characteristics of the prepared controlled-release fertilizers (CRFs) in soil, 5 g of each CRF was enclosed in a polypropylene mesh bag. The treatments included T1 (pure urea), T2 (starch-coated urea), T3 (neem-oil-coated urea), and T4 (gum-acacia-coated urea). These CRF-filled mesh bags were buried at a depth of 10 cm in individual pots containing 100 g of soil, with soil moisture set at 50% of field capacity. The pots were then incubated at 25°C in a controlled environment. This experiment followed a completely randomized design (CRD) with three replicates. Mesh bags were retrieved at intervals of 1, 3, 7, 14, 28, and 35 days during the incubation period. Upon retrieval, the mesh bags were rinsed with distilled water to remove soil particles, and their contents were analyzed for total nitrogen content using the Kjeldahl procedure (Van Schouwenberg and Walinge, 1973).

Pre-Soil Sampling and Analysis

Soil sampling was done at the start of the experiment. The samples were collected at a depth of 0- 20 cm from the University Research Farm. The soil was dried, ground, and sieved through a 2 mm sieve. The physicochemical properties of soil including soil texture (Gee and Bauder 1986), pH and EC (McLean 1983), Total organic carbon (Nelson and Sommers 1996), total Nitrogen (Bremner and Mulvaney, 1983), NO₃-Nitrogen were determined by Salicylic acid method (Vendrell and Zupancic 1990), NH₄-N (Anderson and Ingram 1994), Olson phosphorus (Olsen 1954) and extractable potassium (Helmke and Sparks 1996).



Figure 1. Lab incubation experiment for nitrogen release pattern in soil

Pot Experiment

To assess the agronomic effectiveness of Controlled-Release Fertilizers (CRFs), a pot experiment was conducted. The experiment featured four treatments: T1 (control, no fertilizer), T2 (starch-coated urea), T3 (neem oil-coated urea), and T4 (acacia gum-coated urea). The experiment followed a Completely Randomized Design (CRD) with three replications. Five-kilogram pots were filled with air-dried soil, and each treatment was incorporated into the soil at the recommended nitrogen rate for okra cultivation. Okra seeds (variety: Sabzpari) were sown, maintaining soil moisture at field capacity. Following germination, two seedlings per pot were retained for growth. Pots were regularly irrigated with distilled water to maintain soil moisture at field capacity. At the stage of the first true leaves (hag leaf stage), plant height was measured. Upon crop maturity, harvest was conducted, and data on fresh shoot weight were collected. Crop samples were subsequently oven-dried at 60°C until reaching a constant weight, and dry shoot weight was recorded. Plant samples were processed, milled, and analyzed for total nitrogen content, while soil samples were analyzed for NH₄-N and NO₃-N levels.

Plant Analysis

Biomass Yield

Two months post-maturity, two randomly selected okra plants were uprooted from the pots, and their fresh weights were recorded. Subsequently, the two plants were subjected to drying in an oven at 100°C for three days until complete dryness. The dry matter weight was then measured and recorded. Biomass yield was determined by weighing the dried samples, and the resulting data were recorded.

Total Nitrogen

Total N was calculated by using a spectrophotometer at the wavelength of 665 nm, ([Bremner and Mulvaney, 1983](#))

Statistical analysis

Statistical analysis was carried out to check the significance of treatment means using the Fisher Analysis of Variance method and treatment means (main effects and interaction) were differentiated by applying the Least Significant Difference test (LSD) at 5% probability level (d Steel and Torrie 1986) utilizing Statistics software.

Results and discussion

The daily temperature (°C) and rain (mm) were measured throughout the research period the highest rainfall was 100 mm and the temperature was 30 Co

while the lowest rainfall was 15 mm and the temperature was 5°C (fig 2).

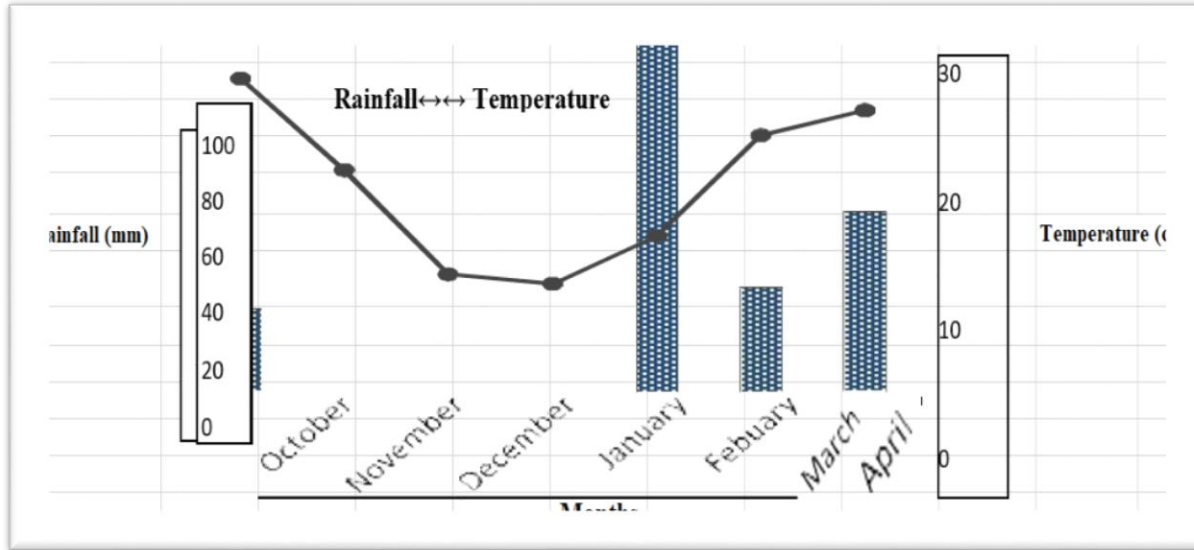


Figure 2 data for temperature and rainfall of related months

Physio-chemical properties of soil under observation and other parameters are given in table 1.

Table 1: Physio-chemical properties of the soil

Sand (%)	Silt	Clay	Texture	Moisture (%)	pH	EC (dSm-1)	Total nitrogen	Nitrate nitrogen mgkg-1	Olsen phosphorus mg kg-1	Extractable potassium mg kg-1
38.10%	32.02%	29.06%	clay loam	17%	7.7	0.2	6.63%	5.5	3.8	130

Plant parameters ammonium nitrogen

The results regarding treatments of polymer-coated urea and conventional urea in comparison with the control (no fertilizer) are presented in Figure 3. The concentrations of ammonium nitrogen in all polymer-coated treatments were low as compared to the conventional urea. However, the concentration of ammonium nitrogen increased gradually in different polymer-coated urea and conventional urea treatments as compared to the control, but their release rate varied with the N source. It was observed that a relatively rapid release of NH₄-N was noticed in starch-coated urea i.e. 4.807, 10.57, 13.54, 20.213, 30.213, and 42.113 (ug per gram) at day one, three, day seven, day fourteen, day twenty- one, day twenty-eight and day thirty-five, in increasing order and polymer-coated neem oil 3.747, 6.31, 9.217, 16.213, 24.44 and 30.327(ug per gram) at day 1, 3, day 7, day 14, day 21, day 28, and day 35. In polymer-coated acacia gum, the release rate was 2.5, 4.55, 7.103, 10.123, 15.23, and 19.027(ug per gram) on day one, three, day seven, day fourteen, day twenty-one, day twenty-eight and day thirty-five respectively. Concentrations of ammonium nitrogen for conventional urea were 6.693, 11.323, 14.943, 24.213, 33.467, and 46.313(ug per gram) at day one, three, day seven, day fourteen, day twenty-one, day twenty-eight and day thirty-five, in increasing order respectively. Among all these three polymers i.e.,

starch, neem oil, and acacia gum, acacia gum shows the best results and slows the process of concentration of nitrogen losses in soil. It means that the use of polymer is essential to reduce the loss of nitrogen which is an essential nutrient for plant growth and development. The concentration of conventional urea was observed higher than the polymers coated urea because of its rapid N release property. According to statistical analysis, the results were significant. The hydrolysis process of urea is relatively less fast in the acacia gum-coated urea fertilizer than in the conventional urea and other two polymers coated urea due to the coatings of polymers material over the urea fertilizer (Junejo et al. 2011). The prolonged availability of urea to plants due to the delayed solubilization of urea slows nitrification, which reduces nitrate leaching and denitrification. By reducing ammonia volatilization, controlling N immobilization, delaying nitrification, and reducing losses from leaching and denitrification, urea coating also helps to improve nitrogen use efficiency (NUE). Through a regulated nitrogen release into the soil solution, polymer-coated urea offers a more precise and effective means to fulfill plant nitrogen requirements. As stated by (Hopkins et al., 2008), one of the main objectives is to improve production and efficiency while reducing excessive nitrogen loss through leaching and denitrification. By minimizing labor-intensive fertilizer application and improving

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urea fertilizer management, this is accomplished. The conversion of R-NH₂ into gaseous ammonia (NH₃) and subsequent conversion into ammonium (NH⁺) is known as ammonification. This process is aided by the water extraction from urea fertilizer as

well as the breakdown of soil organic matter and microbial residues. However, gaseous ammonia often dissipates quickly into the environment, particularly when ammonification occurs near to the soil surface (Schlesinger and Hartley 1992).

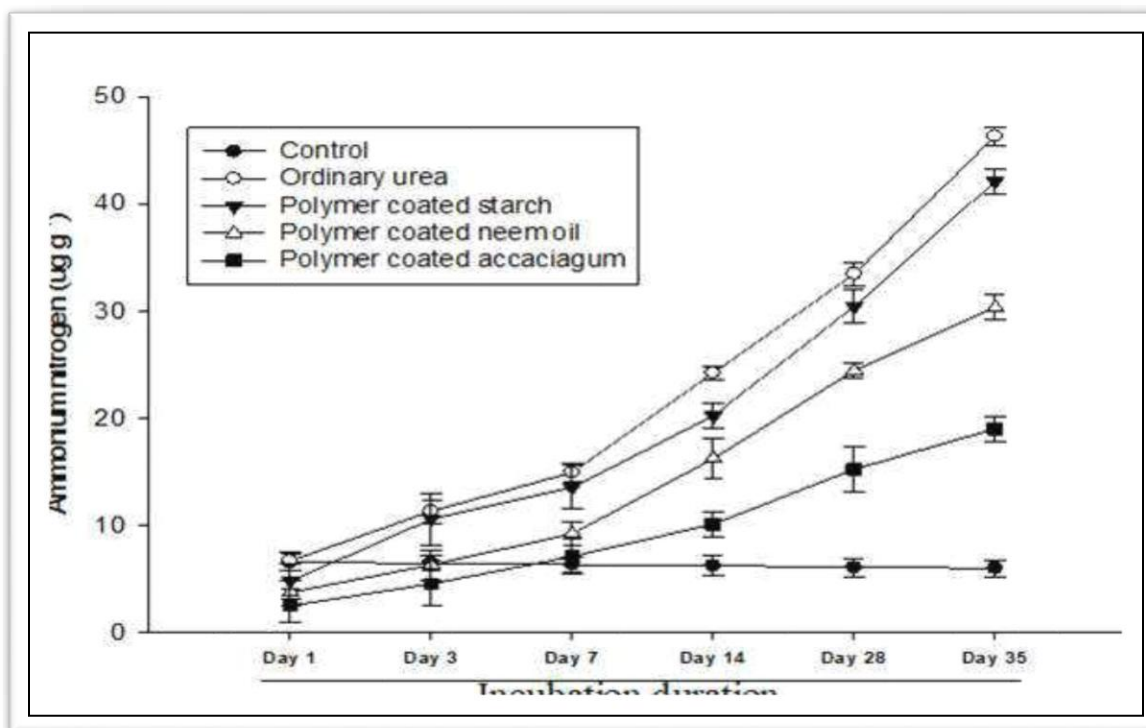


Figure 3: Ammonium-nitrogen released from PCUs and conventional urea at different time intervals during the incubation

Under certain situations, ammonium (NH₄⁺) in the soil can be stable, but in other soil environments, it rapidly nitrifies, converting to nitrate (NO₃⁻). The high solubility of nitrate and its attraction to soil colloids make it quickly lost by leaching. Additionally, it is capable of denitrification, which, particularly in low-oxygen environments, releases nitrous oxide (N₂O) into the atmosphere. Nitrification adds to nitrogen loss as N₂O. Nitrogen deficit in plants can result from several loss pathways and inadequate fertilization. Chlorosis, stunted development, increased susceptibility to infections and pests, and finally plant death are all consequences of insufficient nitrogen, which also impacts the formation of chlorophyll and proteins (Marschner 2012).

Nitrate Nitrogen:

The results regarding NO₃-N in different polymer-coated urea, conventional urea, and control are presented in Figure 4. The concentration of nitrate-nitrogen was increasing gradually in all (PCUs) and conventional urea as compared to control (no fertilizer) but their release rate was different. As the fertilizer made up of different polymer coatings is a

slow-releasing fertilizer its release rate is slower than that of conventional urea. On day 1 the concentration of nitrate-nitrogen in conventional urea was 6.78 (ug g⁻¹), in starch, it was 6.42 (ug g⁻¹), in neem oil 6.12(ug g⁻¹) and in acacia gum polymer coated treatment it was only 2.113 (ug g⁻¹), while in control conditions it was 6.21 (ug g⁻¹). At day 3, the concentration of nitrate-nitrogen in conventional urea was 8.92 (ug g⁻¹), in starch coated urea treatment 7.32 (ug g⁻¹), in neem oil it was 6.88(ug g⁻¹), while acacia gum treated still shows very low concentration i.e. 2.69 (ug g⁻¹), whereas in control conditions it was 6.30(ug g⁻¹) on day 3. At day 7, 12.86 (ug g⁻¹) concentration was recorded in conventional urea whereas in PCUs i.e., starch, neem oil, and acacia gum it was 10.06 (ug g⁻¹), 9.96 (ug g⁻¹), and 4.88 (ug g⁻¹) respectively, and in the control treatment, it was 6.24 (ug g⁻¹). At the interval of day 14, the concentration was increased to 6.19 (ug g⁻¹) in control conditions, and in polymer-coated starch urea it was 17.40(ug g⁻¹), in polymer-coated neem oil it was 16.03 (ug g⁻¹) and in acacia gum (PCU) shows very low concentration i.e., 6.78 (ug g⁻¹), while the concentration of conventional urea was

21.86 (ug g⁻¹). On day 28 the concentration was increased up to 30.12 (ug g⁻¹) in conventional urea and in starch (PCU) it was increased to 25.31 (ug g⁻¹) as well as in neem oil (PCU) the recorded concentration was observed at 23.02 (ug g⁻¹) and in acacia gum coated urea it gradually increases to 11.62 (ug g⁻¹) and in control conditions, it was 6.117 (ug g⁻¹) observed. There was a gradual increase in the concentration at day 35, the higher concentration was observed with the value 41.29 (ug g⁻¹) in conventional urea while, in control conditions it was 5.87(ug g⁻¹) in starch-coated urea the amount was higher up to 30.31(ug g⁻¹), in neem oil (PCU) the recorded amount was 27.13(ug g⁻¹) and in acacia gum polymer coated urea gradually increases to 18.26 (ug g⁻¹) which indicates the slow releasing process of nitrate nitrogen concentration among all the three different coated polymers. It was observed with the value 18.26 (ug g⁻¹) in acacia gum polymer-coated urea and the concentration was higher in conventional urea at 41.29 (ug g⁻¹). This showed an ascending order while the concentration in control was 6.21 on day 1 and the concentration on day 35 was 5.87 (ug g⁻¹) showing a descending order and it was much lower than the other treatments. The release rate of conventional urea was higher as compared to polymer-coated urea, while the chances

of losses of fertilizer are lesser in acacia gum polymer-coated urea. Based on statistical analysis, the results are significant. A study by (Junejo et al., 2011) showed that polymer-coated urea releases nutrients more slowly than untreated urea. Reddy (1999) investigated several coated urea types and discovered a 38-40% reduction in NH₄-N loss, contributing to lower nitrogen loss in the environment. According to research by (Yaseen et al., 2006) urea with a polymer coating and regular urea both improved soil NO₃-N component concentrations above NH₄-N component concentrations for up to 6 weeks. Because it is expensive and environmentally harmful, addressing nitrogen loss from fertilizers is essential. Efforts have been made to improve nitrogen fertilizer efficiency, and utilizing CaC₂ as a nitrification and de-nitrification retarder can efficiently keep nitrogen in NH₄-N form, boosting fertilizer efficiency. Through the maintenance of NH₄⁺ levels and the reduction of nitrogen losses through denitrification and NO₃ emission, nitrification inhibitors have been certified for increasing crop yields. When employing traditional nitrogen sources at regular rates, studies have shown that NO₃-N leaching for potatoes on sandy soils dramatically increased from 71 to 257 kg N ha⁻¹.

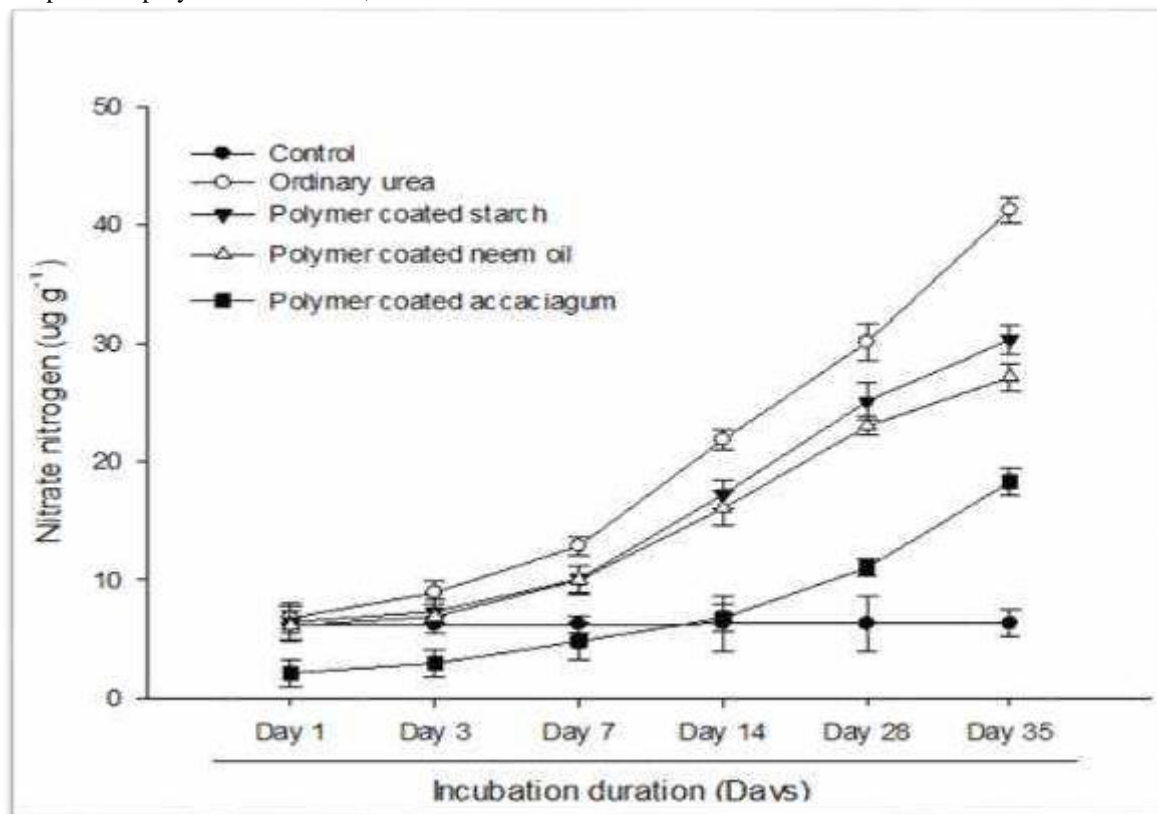


Figure 4: Nitrate-nitrogen released from PCUs and conventional urea at different time intervals during the incubation

But when controlled-release fertilizers like PCU and SCU were used, NO₃-N leaching was significantly decreased to 7-62 kg ha⁻¹ and 13-36 kg ha⁻¹, respectively, especially when spray irrigation was used. These findings support the use of controlled-release fertilizers to reduce NO₃-N leaching.

In contrast to previously reported levels at equal N application rates, the leaching of NO₃-N with solvent N consistently showed lower values under the current researching conditions. However, it is notable that our results were within the lower range of NO₃-N leaching related to Polymer-Coated Urea (PCU) application as recorded in the current literature. Several variables may have contributed to this result, including unusual dry weather conditions that reduced total water circulation through the soil. The majority of prior conducted research employed N during the growth stage or later, in line with current best management practices intended to reduce NO₃ leaching, as opposed to several prior studies that applied N fertilizer at the time of planting (Gasser et al., 2002). According to studies on the cultivation of potatoes in sandy soils, using most of the N fertilizer after the tuber initiation stage helps to reduce NO₃-N leaching (LeMonte et al., 2009)

Effect of coated urea on N uptake by okra plants Total Nitrogen

Table 2: N concentration and uptake by okra plant

Treatment	N conc. (mg g ⁻¹)	N uptake (g/plant)	N uptake (g/pot)
Control	0.133	0.0038	0.016
Conventional Urea	0.372	0.0108	0.036
Starch coated Urea	0.399	0.0129	0.039
Neem oil coated urea	0.412	0.141	0.042
Acacia gum coated urea	0.425	0.149	0.049

Effect of Polymer Coated Urea and conventional urea on Plant biomass

The average plant height for starch-based polymer-coated urea (PCU) was 87 cm, with an average shoot fresh weight of 78 grams per plant and an average shoot dry weight of 20 grams per plant (table 3). Nitrogen-use efficiency (NUE) was 48% for plants grown in starch PCU-treated soil (table 3). The mean plant height for neem oil-based PCU was 98 cm, with an average fresh shoot weight of 84 grams per plant and an average dry shoot weight of 22 grams per plant. For PCU made from acacia gum, the average plant height was 113 cm, and the average fresh shoot and dried shoot weights were 92 grams and 24 grams, respectively. NUE was 49% for neem oil PCU-grown plants and 52% for acacia gum PCU-grown plants. With a NUE of 41%, conventional urea-produced plants with a lower average height of 76 cm, an average fresh shoot weight of 70 grams per plant, and an average dry shoot weight of 17 grams per plant. The average plant height in the

In contrast to traditional urea, the results of this investigation showed that coating urea with three different polymer materials significantly reduced ammonium loss, increased nutrient absorption, and enhanced plant development (table 2). Acacia gum polymer-coated urea, at 0.049g per pot, had the maximum nutrient absorption, followed by neem oil polymer-coated urea at 0.042g per pot and starch polymer-coated urea at 0.039g per pot. Nutrient absorption by okra plants from traditional urea-treated soil, on the other hand, was lower, at 0.036g per pot, while the control group had the lowest uptake at 0.016g per pot. Statistical analysis confirmed the importance of these findings. Nasima et al., (2010) reported that the application of polymer-coated urea treatments increased not only dry matter production but also plant nitrogen absorption. This rise in yield can be related to the physiological effects on the growth of plants and a reduced rate of urea hydrolysis in the soil, resulting in to reduced nitrogen fertilizer loss. Slow-release urea formulations that modify urea release from granules have been developed to lessen these losses. According to (Yu et al., 2010), polymer-coated urea, functioning as a controlled-release nitrogen source, can elevate plant nitrogen content, minimize nitrogen losses, optimize nitrogen use efficiency, and retain more nitrogen in the vicinity of the roots.

control was 65 cm, with an average fresh shoot weight of 59 grams per plant and an average dry shoot weight of 12 grams per plant.

Significant results are drawn via statistical analysis. Conventional urea has a more rapid release rate, resulting in fertilizer waste due to volatilization and leaching, limiting nutrient availability and affecting plant production. Acacia gum polymer-coated urea stands out as a slow-release fertilizer, reducing environmental losses while sustaining continuous plant availability throughout development phases. The increased nitrogen availability is attributable to the better plant growth observed with Acacia gum polymer-coated urea-treated soil. In line with other investigations, this slow-release strategy minimizes ammonium and nitrate losses (Junejo et al., 2009). Compared to conventionally treated soil, plants in the polymer-coated urea-treated soil exhibit higher nitrogen absorption. Along with reducing nitrogen loss, the better coating has a good impact on physiological plant processes.

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Table 3: Effect of PCUs and Conventional urea on biomass of okra plant

Treatment	Plant height(cm)	Fresh shoot weight (g plant-1)	Dry shoot weight (g plant-1)
Control	65 e	59 e	12 d
Conventional urea	76 d	70 d	17 c
Starch coated urea	87 c	78 c	20 b
Polymer-coated neem oil	98 b	84 b	22 a
Polymer coated acacia gum	113 a	92 a	24a

Significant height differences were seen between treatments, but results for biomass remained ambiguous. Higher nitrogen treatment is expected to increase biomass, which is consistent with previous studies (Walker et al., 2007). Nitrogen's function in supporting both vegetative and reproductive development, as well as the formation of energy-efficient compounds managing food production and plant assembly, can be associated with the large vegetative biomass effects (WU FB and Xu, 1998). According to (Gairola et al., 2009) nitrogen plays a major role in plant growth as a vital component of chemical and biological molecules, having a significant impact on both food production and overall plant functions.

Conclusions

Higher ammonium-nitrate and nitrate nitrogen concentrations in soils treated with polymer-coated urea, with acacia gum-coated urea showing the slowest release. As release rates varied over time, nitrogen levels increased. Polymer-coated urea reduced ammonium loss, increased nutrient absorption, and boosted plant growth compared to conventional urea. Urea with acacia gum coating functioned well, leading to increased nitrogen absorption. In conclusion, biodegradable polymer-coated urea increases nitrogen usage efficiency, decreases losses, and enhances plant development, particularly when combined with acacia gum-coated urea. These results have practical significance for agricultural sustainability and environmental preservation.

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Conflict of interest

There is no conflict of interest among the authors of the manuscript.



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