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Synthesis, Characterization and Impact of Nano-urea on Growth and Yield of Wheat in Inceptisol

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

A study on "Synthesis, Characterization and Impact of Nano-urea on Growth and Yield of Wheat in Inceptisol" was conducted at Division of Soil Science, College of Agriculture, Pune during *rabi* 2021. The object of this experiment is to synthesize and characterize nano-urea at post graduate laboratory of Soil Science, College of Agriculture, Pune and which is named as COAP (College of Agriculture, Pune). Nano-urea was synthesized from granular conventional urea and characterised for size of nano particles by using scanning electron microscope. The experiment consisted of twenty one combinations of treatment based on 3 levels of recommended doses of nitrogen (0, 50, 75%) and six nitrogen levels for foliar sprays and water spray as a control which was replicated twice in factorial completely randomized Design (FCRD). The foliar sprays of nitrogen consisted of four levels of COAP nano-urea @ 50, 100, 150 and 200 ppm, IFFCO nano-urea @ 160 ppm and conventional urea @ 10,000 ppm applied at 30 and 50 DAS.

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It could be revealed that average size of COAP (22.419 nm) and IFFCO (22.773 nm) nano-urea was almost same. Results revealed that conjoint application of 75% nitrogen along with two foliar sprays of conventional urea @10,000 ppm, COAP nano-urea @ 200 ppm and IFFCO nano-urea @ 160 ppm taken at 30 and 50 DAS for wheat recorded significantly higher periodical plant height and leaf area at 35 and 55 DAS. Significantly higher plant height was reported with combine application of 75% RDN along with two foliar sprays of urea @ 10,000 ppm (76.83 cm) which was statistically at par with nano-urea of IIFCO (76.20 cm) and COAP (74.20 cm) than rest of the treatment at 55 DAS. In case of leaf area, application of 75% RDN along with two foliar sprays of either conventional urea @ 10,000 ppm, IFFCO nano- urea @ 160 ppm and COAP nano-urea @ 200 ppm at 35 and 55 DAS. Application of 75% recommended dose of nitrogen along with two foliar sprays of 10,000 ppm (44.91 and 62.87 g pot⁻¹) and 75% RDN + COAP nano-urea @ 200 ppm (44.83 and 60.66 g pot⁻¹).

Keywords: Nano-urea; urea; foliar spray; chlorophyll; grain yield wheat.

1. INTRODUCTION

Synthetic chemical fertilizers are used for the optimal growth and productivity of crops, but, at present, adopted agricultural practices have not been particularly successful to enhance plant nutrient uptake, nutrient use efficiency (NUE) and crop productivity [1]. In most cases, synthetic fertilizers used in extensive agriculture have low NUE values [2]. NUE of three most basic i.e., nitrogen, phosphorus, nutrients. and potassium, are low as 30 to 35, 18 to 20, and 35 to 40 percent, respectively. The effectiveness of fertilizer N recovery by the first crop is 30 to 50 percent. The leftover N is either retained in the soil, with very limited recovery in subsequent crops (7 percent of applied N for up to six crops), or it is lost from the soil-plant system, producing major ecosystem changes. To reduce NO₃-N leaching, producers are advised to reduce or completely eliminate summer fallow (particularly tilled) by increasing cropping frequency using no tillage. The key to reducing NO₃- N leaching and maintaining crop production is to avoid excessive water leakage by controlled irrigation and to match N application to crop nitrogen needs in both time and space.

Further more than half of the broadcasted fertilizers in the field are lost and do not reach their targeted sites due to different factors such as hydrolysis, leaching, microbial immobilization and degradation. A low NUE can lead to the intensive use of synthetic fertilizers to increase crop production [2]. However, in the long term, this intensive application of synthetic fertilizers can result in severe environmental risks such as air pollution, soil degradation, water eutrophication, and groundwater pollution [3].

Furthermore, the over and imbalance application of synthetic fertilizers increases the cost of their production and decreases the profit margin of farmers. Low NUE values and increased environmental risks related to the use of more synthetic fertilizers has been a long-term limitation to achieve sustainability in agriculture. The use of urea, DAP, and MOP has been found to reduce fertilizer efficiency by 20 to 50 percent for nitrogen, 10 to 25 percent for phosphorus, and 70 to 80 percent for potassium. [4] Attributable to urea leaching losses, in addition to volatilization and denitrification losses, which contribute not only to green- house gas emissions but also to specific health dangers such as blue baby syndrome.

Therefore, sustainability in agriculture can be achieved through the implementation and utilization of innovative techniques that could enhance global food production with protecting natural resources [5]. India is the 3rd largest fertilizer user with average rate of nutrient application is 96 kg ha⁻¹. This consumption is highly concentrated in certain areas and large area received very little fertilizer. Out of 718 districts (sub unit affected), 26% of total fertilizer is consumed in 180 districts, 50% in 270 districts and 75% in 268 districts [6].

In order to address issues of low fertilizer use efficiency, imbalanced fertilization, multi- nutrient deficiencies reducing low response ratio and decline in soil organic matter, it is indeed need of the day to evolve the nano-based fertilizer formulations with multiple functions [7]. Nano fertilizer technology is designed to distribute nutrients in a controlled pattern in response to crop need, improving nutrient use efficiency while avoiding negative side effects [8]. Nano-fertilizers are more efficient than traditional fertilizers because they can regulate the flow of nutrients based on the needs of the crops.

fertilizers are crucial technique Nano in agriculture for improving crop growth, yield, and quality metrics, as well as reducing fertilizer waste and cultivation costs. Nano fertilizers are particularly useful in precision agriculture for accurate nutrient management, since they match the crop growth stage for nutrient and may give nutrient throughout the crop growth period. Nano fertilizers enhance the surface area available for various metabolic processes in the plant, resulting in a faster rate of photosynthesis and increased dry matter and crop production. It also protects the plant from biotic and abiotic stressors.

Nano-fertilizers are fertilizers that are encapsulated inside nano porous materials, coated with thin polymer films, or delivered as particles nanoscale or emulsions. Nanotechnology is the revolutionary technology where the particle size ranges between 1 and 100 nm at least in one dimension. Due to their high surface area and high reactivity better penetration into the cell, these can activate plant and microbial activities resulting in more nutrient use efficiency. Nanoparticles mav triaaer enzymes and polysaccharide release and act as effective catalysts in plant and microbial metabolism. Nanotechnology based products and their applications in agriculture may include nano-nutrients, nano pesticides, nano-scale carriers, nano sensors, nano chips, nano cellulose, nano barcode, quantum dots, etc. [7]. The advantages and beneficial effects of nanoparticles and nano-fertilizers were elaborated by Seleiman et al., [1].

Application of fertilizer nutrient through foliar application is always superior than soil application but foliar applied fertilizer nutrient are facing several structural barrier, because the nutrients are salt based (cation and anion) which may struggle to penetrate the plant tissue cells. This is because of pore size of cell wall that range between 5 - 100 nm [9]. Hence, nanoparticle aggregate with diameter less than pore size of plant cell wall which can easily enter through the cell wall and reach up to the plasma membrane [10].

In case of wheat plants nano-particles were present in phloem tissues after foliar application

which means that nano-particles were absorbed and transported through phloem route from leaves to stem down to roots, which was documented with transmission electron microscope [11]. Further [12] also reported nanoparticles with diameter of 100 nm can easily penetrate through the stomata of the leaves and were redistributed from leaves to stem through the phloem sieve.

The experiment is planned with an objective to synthesize nano-urea and its characterization with scanning electron microscope (SEM). The synthesized nano-urea and urea will be assessed by foliar application on wheat at critical growth stages to ascertain their use efficiency. Therefore an experiment was conducted for the "Synthesis and characterization of nano-urea and its effects on growth and yield of wheat in Inceptisol".

2. MATERIALS AND METHODS

2.1 Synthesis and Characterization of Nano-urea

The required quantity of nano-urea was synthesized from laboratory grade granular urea at Division of Soil Science, College of Agriculture, Pune. IFFCO nano-urea was procured from the market. Synthesis of nano urea was carried out by using chemical method as precipitation of urea with tri sodium citrate. Urea @ 0.005 mol was mixed with 5% of 10 ml tri-sodium citrate. The mixture was thoroughly mixed and heated at 70°C for 45 min. Further this mixture was stirred for 48 hours on magnetic stirrer at 45°C. Thereafter ash colour appears as confirms presence of nano-urea. it The synthesized nano-urea was entitled as COAP nano-urea (College of Agriculture, Pune). Both COAP and IFFCO nano-urea were analyzed for size on scanning electron microscope (SEM) at Instrumentation Division, Savitribai Phule Pune University, Pune. Scanning Electron Microscope (SEM) was used for characterization of size of nano particles. It could be revealed from the analytical report of scanning electron microscope the average size of COAP (22.41 nm) and IFFCO (22.77 nm) nano-urea was almost same (Table 1 and Plate 1). The pH and EC of COAP nano-urea in different concentration (from 50, 100, 150 and 200 ppm), IFFCO nano-urea (160 ppm) and conventional urea (10,000 ppm) was found in neutral range (6.56 to 7.11) while EC ranged 0.12 to 1.25 d Sm⁻¹.

Sr. Number	Size of nano	-urea (nm)
	COAP	IFFCO
1	25.59	21.58
2	16.19	21.75
3	30.16	26.57
4	24.87	27.11
5	23.5	29.8
6	17.28	27.11
7	24.43	13.76
8	21.75	16.41
9	24.28	21.89
10	16.14	21.75
Average	22.41	22.77

Table 1. Characterization of nano-urea for size



Plate 1. Crystalline form of COAP Nano-Urea

2.2 Pot Culture Experiment

Pot culture experiment was conducted with twenty one treatment combinations with two replications as stated in Table 3. The soil used in the experiment was grouped under Inceptisol order which comprises dominance of montmorillonite clay with depth 50-60 cm. The required quantity of Inceptisol soil was collected from the Agronomy Farm of College of Agriculture, Pune. The important physicochemical properties of soil were determined by using standard methods and stated in Table 2. There were 42 plastic pots with 140 cm diameter and 40 cm height were used for this experiment. Further processed soil after drying in shade ponding with wooden mortar and pestle and passed through 2 mm sieve @ 25 kg in each pot were filled. As per the treatment required quantity of urea, single super phosphate and muriate of potash used as a source for nitrogen, phosphorus and potassium for this experiment respectively. Healthy eight seeds of wheat (cv. NIAW 1994) were dibbled equidistantly in each pot. The recommended dose of fertilizers @ 120:60:40 kg N, P2O5 and K₂O were used in this experiment. The fifty per cent nitrogen, 100% P2O5 and 100% K2O were applied as a basal dose and remaining 50% N was applied @ 30 days after sowing. The foliar sprays of nano urea of COAP and IFFCO and conventional urea were carried out at 30 and 50 days after sowing as per the treatment. Further, in control treatment water spraying carried out.

Sr. No.	Soil properties	Value	
1.	pH (1:2.5)	7.8	
2.	EC (dS m ⁻¹)	0.20	
3.	Organic Carbon (%)	0.68	
4.	CaCO ₃ (%)	11.4	
5.	Available nitrogen (kg ha-1)	165	
6.	Available phosphorus (kg ha-1)	21	
7.	Available potassium (kg ha-1)	512	
8.	DTPA- Fe (mg kg ⁻¹)	6.07	
9.	DTPA- Mn (mg kg ⁻¹)	6.26	
10.	DTPA- Zn (mg kg ⁻¹)	2.1	
11.	DTPA- Cu (mg kg ⁻¹)	2.3	

Table 2. Initial Soil analysis

Table 3. Treatment details

Factor 'A'	Factor 'B'
N levels through soil	Nitrogen levels for foliar spray
S ₁ : 0% RDN	F ₁ : Water spray
S ₂ : 50% RDN (60 kg N)	F ₂ : 50 ppm through Nano-urea (COAP)
S ₃ : 75% RDN (90 kg N)	F ₃ : 100 ppm through Nano-urea (COAP)
	F ₄ : 150 ppm through Nano-urea (COAP)
	F₅ : 200 ppm through Nano-urea (COAP)
	F ₆ : 160 ppm through Nano-urea (IFFCO)
	F ₇ : 1% (1000 ppm) through Urea
	NOTE:
Growth	stages of wheat for foliar sprays : 30 and 50 DAS

Growth stages of wheat for foliar sprays : 30 and 50 DAS.

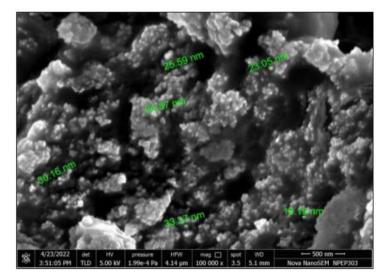
50% RDN was applied at sowing and remaining 50% applied at 20 DAS.

• Basal dose of P_2O_5 and K_2O was applied at the time of sowing.

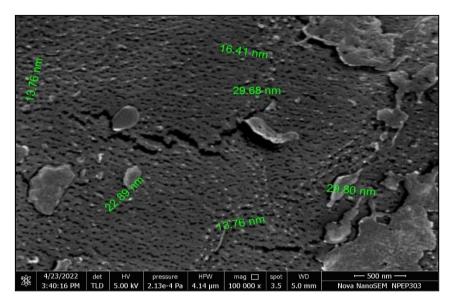
IFFCO Nano-Urea of 500 ml bottle contains 4% N (40,000 ppm nitrogen) and application rate was 2 ml/lit (160 ppm) was used for this experiment.

Plant height and leaf area of wheat in different treatment plots were recorded at 35 and 55 days after sowing. Chlorophyll a, b, c and total were estimated at 35 and 55 days after sowing of

wheat. The grain and straw yield of wheat were recorded in gram per pot. Further the observation and data were processed in factorial randomized block design.



a) COAP



b) IFFCO

Plate 2. Size of Synthesized COAP Nano -Urea

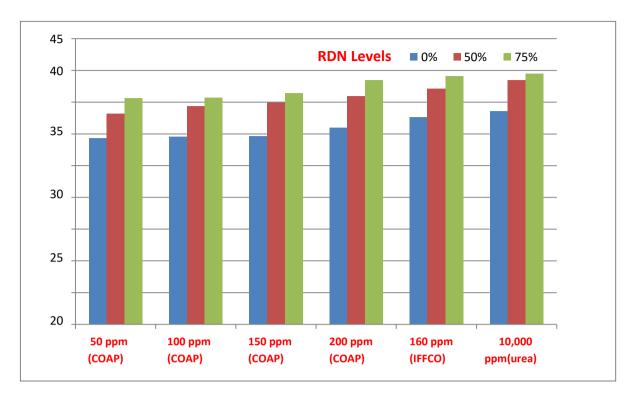
3. RESULTS AND DISCUSSION

3.1 Height of Wheat

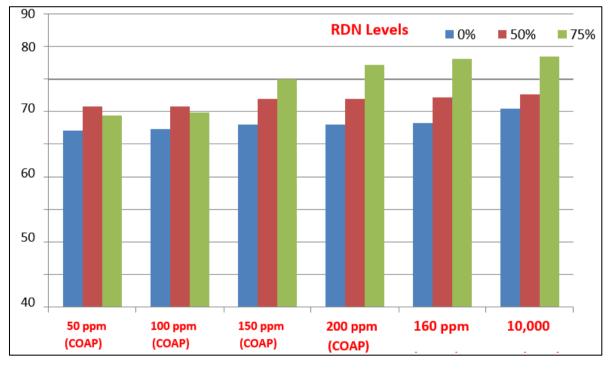
Application of two foliar sprays of nano-urea of COAP (@ 50, 100,150 and 200 ppm) and IFFCO (@ 160 ppm) and convention urea (@ 1%) along with different levels of nitrogen were significantly influenced plant height and leaf area of wheat at 35 and 55 DAS (Table 4 a and b and Fig. 1 a and b). Significantly higher mean height of wheat at 35 (37.01 cm) and 55 DAS (67.31cm) were application recorded with the of 75% recommended dose of nitrogen than rest of the RDN levels. However lower height of wheat was recorded without application of recommended dose of nitrogen. Foliar application of different levels of COAP nano-urea, IFFCO nano-urea and conventional urea at 30 and 50 DAS were significantly affected height of wheat. Foliar Application of conventional urea @10.000 ppm recorded significantly higher height (38.36 cm) of was closely followed wheat which and statistically at par with foliar sprays of IFFCO nano- urea @160 ppm (36.26 cm) at 35 DAS. Foliar sprays of COAP nano-urea @ 50, 100, 150, and 200 ppm reported consistence increase in the plant height of wheat as (32.71, 33.23, 33.68 and 34.06) at 35 DAS respectively. Among the levels of COAP nano-urea (50, 100, 150 and 200 ppm), application of two foliar sprays @ 200 ppm reported significantly higher plant height of wheat at 35 DAS. Foliar sprays of nano-urea from COAP, IFFCO and conventional urea along with RDN levels were reported non-significant results for height of wheat at 35 DAS.

Application of 75% RDN recorded significantly higher mean periodic height of wheat (67.31 cm) at 55 DAS than 50% RDN (63.01 cm) and without RDN (55.18 cm). Significantly higher plant height of wheat was recorded with two foliar sprays of conventional urea @ 10,000 ppm (67.67 cm) taken at 30 and 50 DAS which was closely followed and statistically at par with the application of IFFCO nano-urea @ 160 ppm (65.66 cm) and COAP nano-urea @ 200 ppm (64.73 cm). Increasing trend was noticed in the height of wheat with increasing concentration (from 50 to 200 ppm) of COAP nano-urea application through foliar sprays taken at 30 and 50 DAS.

Combine application of RDN levels @0, 50 and 75% along with foliar sprays of nano- urea showed significant interaction for height at 55 DAS. Significantly higher plant height was reported with combine application of 75% RDN along with two foliar sprays of urea @ 10,000 ppm (76.83 cm) which was statistically at par with nano-urea of IIFCO (76.20 cm) and COAP (74.20 cm) than rest of the treatment at 55 DAS. The increasing trend for the height of wheat was reported with foliar application of nano-urea and conventional urea along with increasing of RDN levels.







b) At 55 DAS

Fig. 1. Effect of foliar sprays of nano-urea and urea along with RDN levels on height of wheat in Inceptisol

Table 4. Effect of foliar sprays of nano-urea and urea on periodic height of wheat in Inceptisol

a) At 35th DAS

NUS	Water Spray	Foliar Sp	rays of COAP	nano- urea	IFFCO Na	no- urea spray	Urea spray	Mean		
		Height (cm)								
		50	100	150	200	160	10,000			
RDN		Ppm	ppm	ppm	ppm	Ppm	Ppm			
0%	28.70	29.30	29.60	29.70	30.20	32.60	34.70	30.68		
50%	30.30	33.20	34.40	34.90	35.15	37.10	39.90	34.99		
75%	34.85	35.65	35.70	36.45	36.85	39.10	40.50	37.01		
Mean	31.28	32.71	33.23	33.68	34.06	36.26	38.36			
	RDN			Nano-urea	l		RDN X Nano-u	rea		
S.E.(m) <u>+</u>	0.46			0.70			1.22			
C.D at	1.38			2.10			NS			
5%										

b) 55th DAS

NUS	Water spray	Foliar Spi	ays of COAP	nano- urea	IFFCO Na	no- urea spray	Urea spray	Mean		
		Height (cm)								
		50	100	150	200	160	10,000			
RDN		Ppm	ppm	ppm	ppm	Ppm	Ppm			
0%	48.30	54.10	54.60	55.90	56.00	56.40	61.00	55.18		
50%	60.60	61.50	61.60	63.80	64.00	64.40	65.20	63.01		
75%	55.50	58.80	59.80	69.90	74.20	76.20	76.83	67.31		
Mean	54.80	58.13	58.66	63.20	64.73	65.66	67.67			
	RDN			Nano-urea	1		RDN X Nano-u	еа		
S.E.(m) <u>+</u>	0.79			1.21			2.09			
C.D at	2.35			3.59			6.23			
5%										

Foliar application of COAP nano- urea and IFFCO nano-urea reported significantly higher height of wheat which might be due to higher absorption by the plants that stimulated the porphyrin molecule of chlorophyll pigment and cytochrome which are essential for photosynthesis and respiration as well as activating enzymes and co enzyme that promotes the production of photosynthates [13].

Mahil and Kumal [14] also reported nano fertilizers have important role in physiological and biochemical process of crops by increasing the availability of nutrients, which helps in enhancing metabolic activities causing higher apical growth and photosynthetic area. Further they also concluded application of nano chelate zinc activity of peroxides enhances catalase. polyphenol oxidase enzyme in cotton and soyabean crops which increases the shoot and root growth. Further, Marimuthu and Surendran [15] also reported foliar spraving of nano formulations increased the plant height and number of branches in black gram. In accordance with Tarafder et al. [16] also concluded that foliar application of zinc-nano fertilizer significantly increased shoot length, root length, root area, plant dry bio mass of pearl millet. Nano particles with the diameter of less than 100 nm can easily penetrate through the stomata of leaves and were redistributed from leaves to stem through the phloem sieve elements. Once the nano particles gets entered into the plant system which may be transported from one cell other cell through to plasmodesmeta and carried by aquaporins, ion channel, endocytosis or by binding to organic chemical [17]. Similar results were also reported by Astaneh et al., [18] and Rathnayaka et al., [19].

3.2 Leaf area of wheat

Leaf area of wheat measured at 35 and 55 DAS was significantly influenced by two foliar sprays of nano-urea from IIFCO. COAP and conventional urea along with levels of recommended dose of nitrogen (Table 5 a and b and Fig. 2 a and b). Leaf area of wheat as influenced by the application of different recommended levels of nitrogen 0, 50 and 75% along with two foliar sprays of COAP nano-urea @ 50, 100, 150 and 200 ppm, IFFCO nano-urea @ 160 ppm and conventional urea @ 10,000 ppm were ranged between 18.76 - 33.74 cm² at 35 and 82.84 cm² -109.12 cm² at 55 DAS.

Results indicated that, application of 75% RDN recorded significantly higher leaf area of wheat at 35 DAS (30.52 cm²) and 55 DAS (104.81 cm²) than rest of the RDN levels. Significantly higher leaf area was reported with the application of conventional urea @ 10,000 ppm (26.65 cm²) which was statistically on par with the foliar sprays of IFFCO nano-urea @ 160 ppm (25.90 cm²), COAP nano-urea @ 200 ppm (25.16 cm²) and 150 ppm (24.75 cm²) at 35 DAS. However similar trend for leaf area was also reported at 55 DAS. Wherein 99.66 cm², 97.88 cm², 96.36 cm² and 95.20 cm² leaf area of wheat was obtained with the application of urea @ 10,000 ppm, IFFCO nano-urea @ 160 ppm, COAP nano-urea @ 200 ppm and @150 ppm respectively which was found to be at par with each other.

Interaction effect among foliar sprays of nanourea from COAP, IFFCO and conventional urea along with recommended nitrogen levels were found significant for the leaf area of wheat at 35 and 55 DAS. However significantly higher leaf area was recorded with the application of 75% RDN along with two foliar sprays of either conventional urea @ 10,000 ppm, IFFCO nanourea @ 160 ppm and COAP nano-urea @ 200 ppm at 35 and 55 DAS.

The particle size of COAP nano-urea (22.41 nm) and IFFCO nano-urea (22.77 nm) applied in the form of foliar sprays which might be the reason for higher leaf area of wheat at 35 and 55 DAS. Lower the size of nano particle has higher surface area along with reactive surfaces which leads to activation of plant enzyme involved in the metabolism. Further, these smaller size nano particles has more ease for plant absorption resulted in the increase in cell division, cell multiplication thereby increase leaf area of wheat [20]. Similar positive results for increase in leaf area of maize with the application of IFFCO nano-urea were also reported by [21]. Further Rathnayaka et al., [19] also concluded foliar spray of nano-urea have higher absorption efficiency through leaves of rice cultivar BG-250. Similar results were also reported by Al-Shammari and Al-Ansari [22].

3.3 Chlorophyll Content in Wheat

Application of recommended nitrogen levels, foliar sprays of nano-urea and their conjoint use were found to be significantly influenced chlorophyll content (chlorophyll a, b and total) in wheat at 35 and 55 DAS (Tables 6 & 7 a, b and c and Fig. 3 & 4 a, b and c).

Chlorophyll a content was ranged from 1.15 to 9.25 mg per gram fresh tissue, Chlorophyll b ranged from 3.03 to 9.2 mg per gram fresh tissue and total chlorophyll from 4.2 -8.55 mg per gram fresh tissue at 35 DAS of wheat (Fig. 3 a, b and c and Fig. 4 a, b and c). Significantly higher chlorophyll a (8.20 mg per gram fresh tissue), chlorophyll b (8.25 mg g⁻¹ fresh weight) and total (7.62 mg g⁻¹ fresh weight) were reported with the application of 75% recommended dose of nitrogen than that of 50% and 0% at 35 DAS. Foliar sprays of urea @ 10,000 ppm reported significantly higher chlorophyll a (6.72 mg g⁻¹ fresh weight) which was found to be at par nano-urea of IFFCO (6.56 mg g⁻¹ fresh weight) and COAP nano-urea (6.30 mg g⁻¹ fresh weight). Application of 75% RDN recorded significantly higher chlorophyll b (8.25 mg g⁻¹ fresh weight) content in wheat than 50% (6.52 mg g⁻¹ fresh weight) and 0% RDN (4.67 mg g⁻¹ fresh weight) at 35 DAS. Foliar sprays of conventional urea @ 10,000 ppm noted significantly higher (7.74 mg g⁻¹ fresh weight) chlorophyll b content which was found to be statistically at par with the foliar sprays of IFFCO nano-urea @ 160 ppm (7.56 mg g⁻¹ fresh weight) and COAP nano-urea @ 200 ppm (7.41 mg g⁻¹ fresh weight) at 35 DAS.

Interaction effect among RDN levels and foliar sprays of nano-urea was also found significant for chlorophyll b content at 35 DAS. Conjoint application of 75% RDN along with two foliar sprays of conventional urea reported significantly higher chlorophyll (9.21 mg g⁻¹ fresh weight) which was found to be statistically at par with the foliar sprays of either IFFCO nano-urea @ 160 ppm (9.07 mg g⁻¹ fresh weight) or COAP nanourea @ 200 ppm (8.83 mg g⁻¹ fresh weight). Total chlorophyll content in wheat at 35DAS reported similar trend of or significantly higher total chlorophyll content (7.65 mg g⁻¹ fresh weight) than rest of the treatment while two foliar recorded sprays conventional urea of significantly higher total chlorophyll (7.10 mg g⁻¹ fresh weight) which was found to be statistically at par with IFFCO nano-urea @ 160 ppm (6.99 mg g⁻¹ fresh weight) or COAP nano-urea @ 200 ppm (6.84 mg g⁻¹ fresh weight).

Chlorophyll a, b and total chlorophyll at 55 DAS in wheat were also significantly influenced by RDN levels, foliar sprays of nano-urea and conventional urea and their combine application

(Table 7 a. b and c). Significantly higher chlorophyll a (3.85mg g⁻¹ fresh weight), b (7.05 mg per gram fresh tissue) and total chlorophyll (7.12 mg g⁻¹ fresh weight) were recorded with the application of 75 % RDN than rest of the treatment. Foliar application of conventional urea @ 10,000 ppm recorded significantly higher chlorophyll a (3.74 mg g⁻¹ fresh weight), chlorophyll b (6.60 mg g⁻¹ fresh weight) and total chlorophyll (6.54 mg g⁻¹ fresh weight). Foliar Application of IFFCO nano-urea @ 160 ppm and COAP nano-urea @ 200 ppm were found at par for chlorophyll a (3.64 and 3.52mg g⁻¹ fresh weight), chlorophyll b (6.48 and 6.40 mg g⁻¹ fresh weight) and total chlorophyll (6.37 and 6.23 mg g⁻¹ fresh weight) with conventional urea @ 10,000 ppm respectively. Higher accumulation of a, b and total chlorophyll in wheat was reported with foliar nano- urea either of COAP or IFFCO which might be due to higher absorption efficiency through leaves.

Seleiman Mahmoud et al., [1] reviewed and concluded that nano fertilizers release their nutrients in 40-50 days while synthetic fertilizers do the same in 4-10 days. As a result of this synthetic fertilizer like N-urea can rapidly lose more than 50% of nutrient contents after field application through leaching and volatization. However nano fertilizers release nutrient as much as 12 times slower than synthetic fertilizer and they can significantly increase the yields and quality traits of crops. The foliar application of nano fertilizers is much better and preferred than soil application of nano fertilizers due to its enhancements significant in growth, physiological and biochemical traits, yield and quality of crops. Further, Rezaei and Abbasi et al., [23] also reported increased fresh and dry weight of cotton due to improved physiological process for chlorophyll synthesis and antioxidant activities. Mahil and Kumal [14] reported nano fertilizers have important role in physiological and biochemical process of crops by increasing the availability of nutrients, which helps in enhancing metabolic activities causing higher apical growth and photosynthetic area. Further they also reported remarkable increase in physiological and biochemical parameters of crop with the application of nano fertilizers. They also summarized that biocompatible magnetic nano fluid had positive influenced on the total chlorophyll content (a and b) in sunflower leaves. Foliar application of COAP nano- urea and IFFCO nano-urea reported significantly higher plant height, leaf area and chlorophyll content might be due to higher absorption by the plants

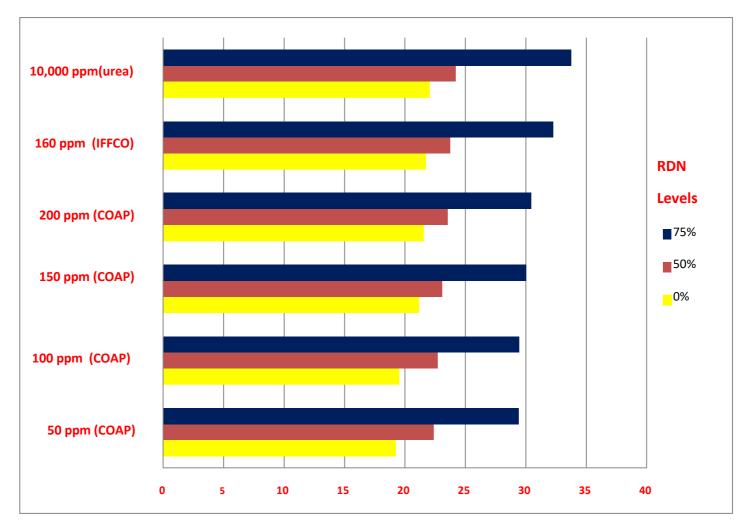
Table 5. Effect of foliar sprays of nano-urea and urea along with RDN levels on Periodic leaf area of wheat in Inceptisol

a) At 35th DAS

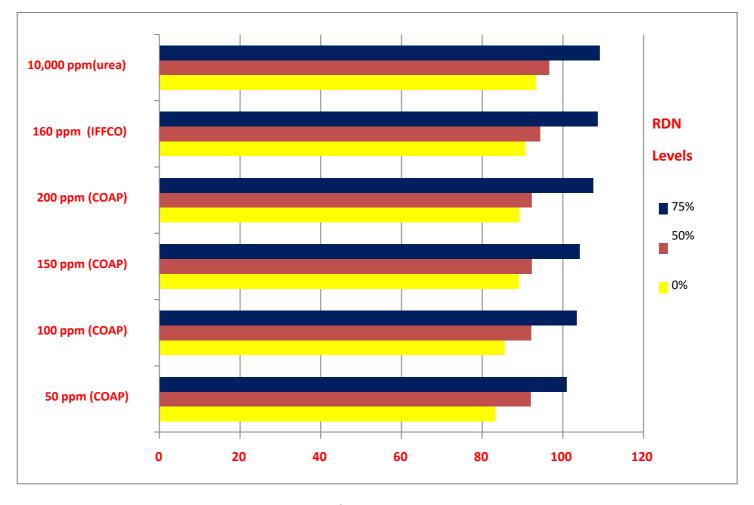
NUS	Water spray	Foliar Sp	rays of COAP	nano- urea		IFFCO Nano- urea spray	Urea spray	Mean				
			leaf area (cm ²)									
		50	100	150	200	160	10,000					
RDN		Ppm	Ppm	Ppm Ppm		Ppm	ppm					
0%	18.76	19.22	19.50	21.15	21.52	21.71	22.02	20.55				
50%	21.78	22.36	22.72	23.08	23.53	23.74	24.20	23.06				
75%	28.32	29.41	29.45	30.02	30.44	32.25	33.74	30.52				
Mean	22.95	23.66	23.89	24.75	25.16	25.90	26.65					
	RDN			Nano-urea	a		RDN X Nano-u	rea				
S.E.(m) <u>+</u>	0.44			0.67			1.17					
C.D at 5%	1.31			2.00			3.72					

b) 55th DAS

NUS	Water spray	Foliar Spray	s of COAP nand	o-urea	IFFCO Nano- urea spray	Urea spray	Mean				
		leaf area (cm ²)									
		50 ppm	100	150	200	160	10,000				
RDN			Ppm	Ppm ppm		ppm	ppm				
0%	82.84	83.29	85.60	89.12	89.19	90.66	93.24	87.70			
50%	91.20	92.11	92.19	92.25	92.29	94.42	96.62	93.01			
75%	99.67	101.00	103.45	104.23	107.61	108.57	109.12	104.81			
Mean	91.23	92.13	93.75	95.20	96.36	97.88	99.66				
	RDN			Nano-urea			RDN X Nano	urea			
S.E.(m) <u>+</u>	1.18			1.81			3.13				
C.D at 5%	3.52			5.38			11.29				



a) At 35 DAS



a) At 55 DAS

Fig. 2. Effect of foliar sprays of nano-urea and urea along with RDN levels on leaf area of wheat in Inceptisol

Table 6. Effect of foliar sprays of nano-urea and urea along with RDN levels on chlorophyll of wheat in Inceptisol

a) Chlorophyll a at 35 DAS

NUS	Water spray	Foliar Sp	rays of COAP	nano- urea		IFFCO Nano- urea Spray	Urea spray	Mean		
		Chlorophyll a (mg g ⁻¹ fresh weight)								
RDN		50 Ppm	100 Ppm	150 Ppm	200 ppm	160 Ppm	10,000 ppm			
0%	1.15	1.53	1.55	1.55	2.00	2.60	2.90	1.89		
50%	4.42	4.56	5.66	7.83	7.86	7.95	8.00	6.61		
75%	6.80	6.95	7.63	8.59	9.05	9.12	9.26	8.20		
Mean	4.12	4.35	4.94	5.99	6.30	6.55	6.72			
	RDN			Nano-ure	а		RDN X Nano-u	rea		
S.E.(m) <u>+</u>	0.11			0.17			0.30			
C.D at 5%	0.33			0.51			0.89			

b) Chlorophyll b at 35 DAS

NUS	Water spray	Foliar Sp	rays of COAP	nano- urea		IFFCO Nano- urea spray	Urea spray	Mean			
			Chlorophyll b (mg g ⁻¹ fresh weight)								
RDN		50 ppm	100 ppm	150 Ppm	200 ppm	160 Ppm	10,000 ppm				
0%	3.03	3.04	4.65	4.90	5.50	5.68	5.88	4.67			
50%	4.27	5.21	5.87	6.36	7.89	7.93	8.13	6.52			
75%	7.23	7.24	7.59	8.58	8.83	9.07	9.21	8.25			
Mean	4.84	5.16	6.04	6.61	7.41	7.56	7.74				
	RDN			Nano-ure	а		RDN X Nano-u	rea			
S.E.(m) <u>+</u>	0.09			0.14			0.24				
C.D at 5%	0.27			0.42			0.73				

NUS	Water Spray	Foliar Sp	rays of COAP	nano- urea		IFFCO Nano- urea spray	Urea spray	Mean
				Т	otal chloroph	ıyll (mg g ⁻¹ fresh v	veight)	
RDN		50 ppm	100 ppm	150 Ppm	200 ppm	160 Ppm	10,000 ppm	
0%	4.20	4.25	4.45	4.64	4.85	5.12	5.25	4.68
50%	3.28	4.90	5.92	6.10	7.41	7.43	7.50	6.08
75%	6.56	6.68	7.25	7.79	8.26	8.44	8.55	7.65
Mean	4.68	5.28	5.87	6.18	6.84	6.99	7.10	
	RDN			Nano-ure	а		RDN X Nano-u	rea
S.E.(m) <u>+</u>	0.06			0.09			0.16	
C.D at 5%	0.18			0.27			0.48	

c) Total chlorophyll at 35 DAS

Table 7. Effect of foliar sprays of nano-urea and urea along with RDN levels on chlorophyll of wheat in Inceptisol

a) Chlorophyll a at 55 DAS

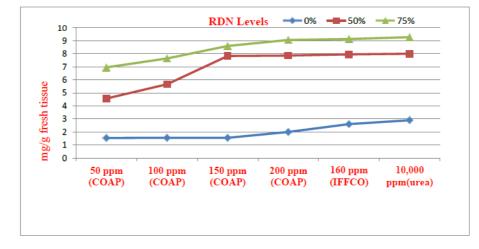
NUS	Water Spray	Foliar Sp	rays of COAP	nano- urea		IFFCO Nano- urea spray	Urea spray	Mean			
			Chlorophyll a (mg g ⁻¹ fresh weight)								
		50	100	150	200	160	10,000				
RDN		ppm	ppm	Ppm	ppm	Ppm	ppm				
0%	0.27	0.31	0.33	0.35	0.74	0.80	0.91	0.53			
50%	1.37	1.45	2.55	3.04	4.20	4.38	4.38	3.05			
75%	1.04	2.66	2.71	3.22	5.63	5.74	5.94	3.85			
Mean	0.89	1.47	1.86	2.20	3.52	3.64	3.74				
	RDN			Nano-ure	а		RDN X Nano-u	rea			
S.E.(m) <u>+</u>	0.05			0.08			0.14				
C.D at	0.16			0.24			0.43				
5%											

b) Chlorophyll b at 55 DAS

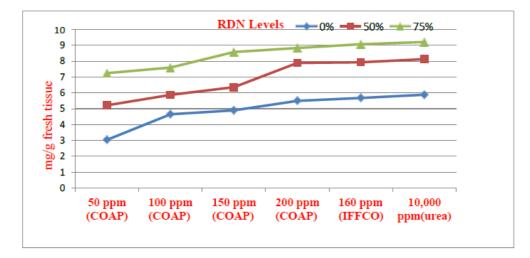
NUS	Water spray	Foliar Sp	rays of COAP			IFFCO Nano- urea spray	Urea spray	Mean		
		Chlorophyll b (mg g ⁻¹ fresh weight)								
		50	100	150	200	160	10,000			
RDN		ppm	ppm	Ppm	ppm	Ppm	ppm			
0%	2.33	3.40	4.13	4.27	5.44	5.46	5.55	4.37		
50%	2.83	2.91	5.16	5.33	6.20	6.38	6.42	5.03		
75%	5.40	6.51	7.16	7.34	7.56	7.60	7.81	7.05		
Mean	3.52	4.28	5.48	5.65	6.40	6.48	6.59			
	RDN			Nano-ure	а		RDN X Nano-u	rea		
S.E.(m) <u>+</u>	0.06			0.10			0.18			
C.D at 5%	0.20			0.31			0.54			

c) Total chlorophyll At 55 DAS

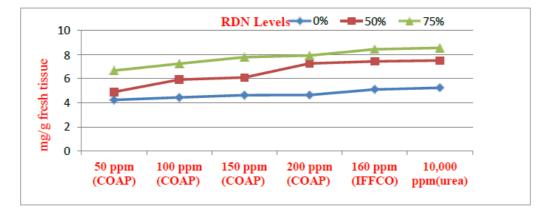
NUS	Water spray		Foliar Sprays	of COAP nano	- urea	IFFCO Nano- urea	Urea spray	Mean				
			spray									
		Total chlorophyll (mg g ⁻¹ fresh weight)										
	\	50	100	150	200	160	10,000					
RDN	\mathbf{i}	ppm	ppm	Ppm	ppm	Ppm	ppm					
0%	3.17	3.64	3.67	3.87	4.77	4.90	4.91	4.13				
50%	3.17	3.48	5.38	5.43	5.97	6.05	6.31	5.11				
75%	4.87	6.01	7.03	7.43	7.95	8.16	8.38	7.12				
Mean	3.74	4.37	5.36	5.57	6.23	6.37	6.54					
	RDN	Nano-urea					RDN X Nano-urea					
S.E.(m) <u>+</u>	0.08			0.12			0.22					
C.D at	0.25			0.38			0.66					
5%												



a) Chlorophyll a at 35 DAS



b) Chlorophyll b at 35 DAS



c) Total chlorophyll at 35 DAS

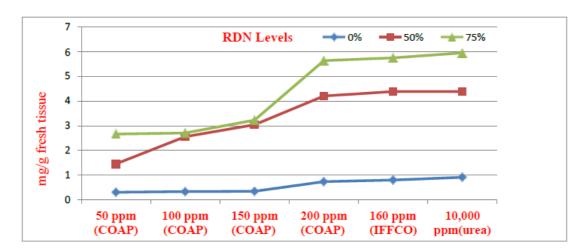


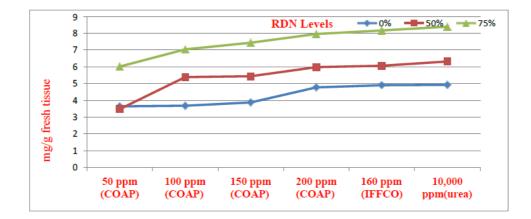
Fig. 3. Effect of foliar sprays of nano-urea and urea along with RDN levels onchlorophyll of wheat in Inceptisol

a) Chlorophyll a at 55 DAS

9 RDN Levels 8 7 mg/g fresh tissue 6 5 4 3 2 1 0 200 ppm 50 ppm 100 ppm 150 ppm 160 ppm 10,000 (COAP) (COAP) (IFFCO) ppm(urea) (COAP) (COAP)

Jadhav et al.; Int. J. Environ. Clim. Change, vol. 13, no. 12, pp. 973-996, 2023; Article no.IJECC.111413

b) Chlorophyll b at 55 DAS



c) Total chlorophyll at 55 DAS

Fig. 4. Effect of foliar sprays of nano-urea and urea along with RDN levels on chlorophyllof wheat in Inceptisol

Table 8. Effect of foliar sprays of nano-urea and urea along with RDN levels on grain and straw yield of wheat in Inceptisol

a) Grain yield

NUS	Water spray	Foliar Sp	rays of COAP	nano- urea	IFFCO Nano- urea spray		Urea spray	Mean		
		Grain yield (g pot ⁻¹)								
		50	100	150	200	160	10,000			
RDN		ppm	ppm	Ppm	ppm	Ppm	ppm			
0%	29.15	30.15	30.85	31.23	32.57	33.05	34.60	31.66		
50%	36.82	37.00	37.80	38.85	39.15	39.57	41.01	38.60		
75%	37.57	38.52	40.37	42.25	44.83	44.91	45.00	41.92		
Mean	34.51	35.22	36.34	37.44	38.85	39.17	40.20			
	RDN	Nano-urea			1		RDN X Nano-u	rea		
S.E.(m) <u>+</u>	0.24			0.37			0.65			
C.D at	0.73			1.12			1.94			
5%										

b) Straw yield

NUS	Water spray	Foliar Sp	ays of COAP	nano- urea	IFFCO Nano- urea spray		Urea spray	Mean		
		Straw yield (g pot ⁻¹)								
		50	100	150	200	160	10,000			
RDN		ppm	ppm	Ppm	ppm	Ppm	ppm			
0%	40.81	42.21	43.19	43.72	45.60	48.29	51.84	45.09		
50%	51.25	51.70	54.60	54.89	55.31	55.39	57.42	54.36		
75%	52.09	53.12	55.11	57.75	60.66	62.87	65.39	58.14		
Mean	48.05	49.01	50.96	52.12	53.85	55.52	58.22			
	RDN	Nano-urea					RDN X Nano-u	ea		
S.E.(m) <u>+</u>	0.42			0.63			1.09			
C.D at	1.22			1.87			3.23			
5%										

which stimulating the porphyrin molecule of chlorophyll pigment and cytochrome which are essential for photosynthesis and respiration as well as activating enzymes and co enzyme which are necessary for the production of photosynthates [13].

3.4 Wheat Grain and Straw Yield

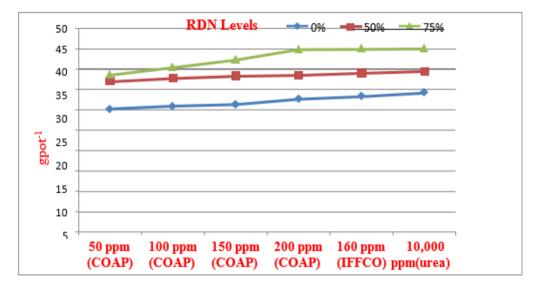
Data presented in Table (8 a and b and Fig. 5 a and b) regarding grain and straw yield of wheat were ranged from 29.15 to 45.00 and 40.81 to 63.00 g per pot and influenced significantly by the foliar sprays of nano-urea of COAP, IFFCO and conventional urea, RDN levels and their conjoint application respectively.

It could be observed from the data that grain and straw yield of wheat was increasing with the increasing levels of RDN as well as levels of COAP nano-urea, IFFCO and conventional urea. Application of 75% RDN recorded significantly higher grain (41.92 g pot⁻¹) and straw (58.69 g pot⁻¹) yield of wheat than 50% RDN (38.15 and 53.42 g pot⁻¹) and 0% RDN (31.61 and 44.25 g pot⁻¹). Foliar sprays of conventional urea @ 10,000 ppm (39.54 and 55.35 g pot⁻¹), IFFCO nano-urea @ 160 ppm (39.06 and 54.69 g pot⁻¹) and COAP nano-urea @ 200 ppm (38.65 and 54.11 g pot⁻¹)were reported significantly higher and on par results for grain and straw yield of wheat respectively. Increasing trend in grain (35.22, 36.34, 37.27 and 38.65) and straw (49.31, 50.87, 52.18 and 54.11 g pot⁻¹) yield of wheat were observed with the foliar application of increasing concentration of COAP nano-urea as @ 50, 100, 150 and 200 ppm respectively.

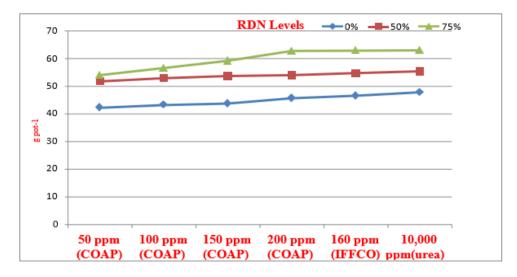
Grain and straw yield of wheat were also found significantly influenced by the combine application of RDN levels and foliar sprays of nano-urea COAP and IFFCO and conventional urea.

Application of 75% RDN level along with two foliar sprays of conventional urea @ 10,000 ppm reported significantly higher grain (45.0 g pot⁻¹) and straw (63.0 g pot⁻¹) yield of wheat which was found to be statistically at par with IFFCO nanourea @ 160 ppm (44.91 and 62.87 g pot⁻¹) and COAP nano-urea200 ppm (44.83 and 62.76 g pot⁻¹) respectively. Spraying of nano-urea at critical growth tillering and jointing stages either of (COAP @ 200 ppm and IFFCO @ 160 ppm) might had increase yield of wheat in Inceptisol due to enhancement of metabolic activity and meristimatic activity.

Size of COAP and IFFCO nano particles was very less (< 30 nm and Table 8) that makes an ease for absorption via foliar resulted in activation of plant enzyme which indirectly plays dominant role in growth and number of tillers for wheat. Foliar application of conventional fertilizers facing several structural barriers to penetrate inner plant tissue cell but nano particles with size less than 50 nm can easily penetrate through stomata of watermelon [12]. Further they also concluded that nano particles with size less than 100 nm can easily penetrate through the stomata of leaves and were redistributed from leaves to stem through the phloem.



a) Grain yield



b) Straw yield

Fig. 5. Effect of foliar sprays of nano-urea and urea along with RDN levels on grain andstraw yield of wheat in Inceptisol

Alzreejawi and Juthery [23] concluded that foliar application of nano particles like nano N, P, K and nano complete micro fertilizer NCM and nano amino acid NAA increase in vegetative growth and yield characteristics of maize. Al Juthery, Estabraq et al.,2020 also observed higher plant growth , chlorophyll, photosynthesis rate , dry matter production and over all plant growth with the foliar application of nano fertilizers for yield and quality for potato.

Rawate et al. [24] concluded that application of 100% RDN along with two sprays of nano-urea at tillering and jointing stage recorded on par results with application of 100% RDN + two foliar sprays of urea at @ 5% for yield of wheat. Rathnayaka et al., [19] also concluded that application of 100% nano fertilizers was found significantly effective for the growth and yield of rice cultivar "Bg 250". Higher leaf area, plant height, chlorophyll content in wheat might be due to higher use efficiency of nano fertilizers that leads reduced losses due to denitrification, volatization and leaching too.

4. CONCLUSIONS

Foliar application of COAP nano-urea @ 200 ppm was found more efficient and complementary with 75% RDN level than only foliar application.Application of 75% RDN along with the two foliar sprays ofnano-urea from COAP @ 200 ppm and IFFCO @ 160 ppm taken at 30 DAS (tillering stages) and 50 DAS (jointing stages) was found superior and statistically at par for plant height, leaf area, chlorophyll, grain and straw yield of wheat grown in Inceptisol. It is necessary to study optimum concentration of nano urea for soil and foliar application to various crops along with growth stages. Further, effect of soil application of nano urea on rhizospheral microflora needs to be studied in future.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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