

Original Research Article

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Performance of Slow Release Hydroxyapatite Coated Urea Nanofertilizer on Aerobic Paddy

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ABSTRACT

Fertilizers, irrigation and high yielding varieties contributed immensely for the success of green revolution. Increasing global food demand necessitates balanced fertilizer usage. Nitrogenous fertilizers considering the losses due to quicker release doesn't synchronize with crop demand resulting in lower crop uptake and use efficiency, necessitating nanoparticle coated urea for sustained production. Hydroxyapatite nanoparticles (HA) and urea coated hydroxyapatite nanoparticles (UHA) was synthesised through wet chemical method, were characterized using Dynamic Light Scattering (DLS), Scanning Electron Microscope (SEM), Powder X-ray Diffraction (PXRD) and Fourier Transform Infrared (FTIR) Spectroscopy. Nitrogen content of UHA particles was found to be 36.5% and diameter of nanoparticles was less than 90 nm. The UHA was further evaluated for its performance on aerobic paddy. Synthesized Nanofertilizer at 25% of recommended dose recorded higher grain yield in aerobic paddy. Nanofertilizer@ 50% dose showed similar performance to 100% dose of conventional urea. The amount of fertilizer application to the environment can be reduced with nanofertilizer compared to regular dosage of conventional fertilizer without compromising the crop yield thereby reducing environmental pollution of water and soil.

Keywords

Hydroxyapatite,
Urea nanofertilizer,
Aerobic paddy,
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Introduction

Conventional fertilizers along with irrigation and high yielding varieties have contributed immensely for green revolution. Intensive agriculture during post green revolution has put extensive burden on soil removing most of the nutrients resulting in nutrient deficient soils. Though the soils have been restored with nutrients time and again using inorganic

fertilizers, farm yard manure and green manures; Indian soils have particularly displayed nitrogen deficiency with 99% of soils responding to nitrogen application. To address this issue and to boost productivity, measures were taken to encourage the use of inorganic fertilizers. As a result, N fertiliser use grew from meagre amount of about 0.45 kg ha⁻¹ in 1950-51, to nearly 87 kg ha⁻¹ in 2014-15 [Chander, 2016].

Conventional bulk fertilizers in general have low nutrient uptake efficiencies necessitating the usage of fertilizers in large quantities. In spite of regular application, available nitrogen in 90% of arable soil is reported to be deficient as nitrogen is subject to excessive losses through volatilization, run off, leaching and denitrification [Manikandan and Subramanian, 2014]. Owing to continuous use of inorganic fertilizers and their susceptibility to undue losses; they have become potential sources to pollute soil, water and air through emission of greenhouse gases and eutrophication. In recent years, efforts are underway to minimize pollution due to fertilizers by altering agricultural practices, using nitrification inhibitors and the use of the new improved fertilizers. Advancements in nanotechnology have opened up novel solutions to address various agricultural problems through synthesis of smart fertilizers which enable plants to absorb nutrients effectively [Raliya, Saharan, Dimkpa & Biswas 2017].

Nanostructured formulations with altered physicochemical properties can be probable solution to the current problems through mechanisms such as targeted delivery or slow/controlled release mechanisms and trigger controlled-conditional release [Guru et al., 2015].

Nanostructured formulations are structured to deliver nutrients to plants allowing controlled or slow release of active ingredients resulting in efficient uptake [Raliya et al., 2017]. Since nanoscale particles are smaller in dimension compared to bulk particles, the plants can absorb them with different dynamics than bulk particles or ionic salts, which presents an added advantage. There is a need for design and synthesis of nanofertilizers to tailor the need of diverse soil, environmental and crops. The use of nanofertilizers is reported to increase fertilizer use efficiency minimizing the potential negative effects associated with

over dosage which reduces the frequency of the application [Kottegoda et al., 2011]. Nanofertilizers mainly delay the release of the nutrients and extend the fertilizer effective period [Naeem et al., 2017]. However, environmental health and safety aspects of nanotechnology should also be considered, and it is crucial to determine the toxicity / biocompatibility of nanofertilizers.

There are many attempts to synthesise, nanocapsules, nanocomposites, nanohybrids mainly to sustain nitrogen release and enhance use efficiency in the form of slow release and controlled-release fertilizers [Shaviv 2000; Tarafdar and Subramanian 2011; Xiaoyu et al., 2013; Manikandan & Subramanian 2014; 2016]. N fertilizers with controlled release characteristics have been synthesized using the adsorbents zeolite, halloysite, montmorillonite and bentonite nanoclays [Manikandan & Subramanian 2014]. Kottegoda et al., [2011] have synthesized by encapsulating urea coated hydroxyapatite nanoparticles in wood *Glyricidia sepium* while Subbaiya et al., [2012] have used both chemical and biological method of synthesis. These methods have lengthy process and difficult to scale up, hence a simple method of nanofertilizer production amenable for large scale production and testing its applicability in crop production can help for upscaling this futuristic technology. With this background, research was undertaken to synthesize urea coated hydroxyapatite nanofertilizer for enhanced nitrogen use efficiency and its field testing using aerobic rice as test crop.

Materials and Methods

Synthesis of hydroxyapatite nanoparticles

All the reagents used in this experiment were purchased from HiMedia and of analytical grade. Hydroxyapatite nanoparticle was synthesized using chemical synthesis process

as described by Mateus et al., [2007] using aqueous solution of calcium hydroxide and orthophosphoric acid (H_3PO_4 , 85%). 1L aqueous solution of 1M calcium hydroxide was prepared and 1L of 0.6 M orthophosphoric acid was added drop-wise with continuous stirring on a magnetic stirrer at room temperature. The suspension obtained was washed with deionised water and dried at 100°C for 24- 48 hrs and nanoparticles were characterized using DLS, EDS, PXRD, SEM and FTIR.

Synthesis of urea coated hydroxyapatite nanoparticles

Urea coated hydroxyapatite nanoparticle was synthesised by modifying the method of Kottegoda *et al.*, [2017]. The white solution of hydroxyapatite nanoparticle was added to 100 ml of saturated urea solution (approx. 300g). The suspension was constantly stirred for 12 hours at 1000 rpm at room temperature. The urea-hydroxyapatite solution was allowed to settle and the excess liquid was drained out. The urea modified nanofertilizer was washed and dried at 65°C for one to two weeks. Further, characterization was done using DLS, PXRD, SEM and FTIR.

Nitrogen estimation

The nitrogen content of urea coated HA nanoparticle was determined by Kjeldahl's method. A sample of 0.5 g was digested using concentrated H_2SO_4 and a digestion mixture (containing K_2SO_4 and $CuSO_4 \cdot 5H_2O$ and selenium in the proportion of 100:20:1) and was distilled in alkaline medium. The liberated NH_3^+ was trapped in boric acid and titrated against standard H_2SO_4 as described by [Jackson 1973].

Characterization of synthesized nanoparticles using Powdered XRD, SEM, FTIR

Powder X-ray diffraction patterns of the

synthesized samples were obtained using Rigaku Ultima IV X-ray powder diffractometer using $Cu-K\alpha$ radiation ($\lambda=0.154$ nm) over 2θ range of 10-90°, with a step size of 0.02° and a step time of 2 s. Stability of synthesized nanoparticles were analyzed by measuring zeta potential using Microtac Flex. PXRD and zeta potential analysis was carried out at University of Mysore.

Scanning electron microscopy was done using Carl Zeiss Ultra 55 FE-SEM for morphological analysis and determination of particle size. Fourier Transform Infrared Spectroscopy was done using Perkin Elmer Spectrum v 10.03.09 software to analyze functional groups and molecular bonding of synthesized samples. SEM and FTIR were carried out at CeNSE, Indian Institute of Sciences, Bengaluru.

Experimental design for Pot experiments

The pot experiments were conducted in the College of Agriculture, Mandya during the year 2016-18. The experiment was conducted to differentiate the influence of varying doses of urea modified hydroxyapatite nanofertilizer and recommended doses of conventional urea on growth and yield parameters of aerobic rice varieties BI33 (Anagha) and KMP175 (Daksha).

The growth medium used was sandy loam soil. The soil was collected from completely pulverized plot and was processed by removing stone and stubbles. The soil was completely mixed and soil samples were drawn. After mixing the soil, each pot was loaded with 7 kg of soil and was watered.

The soil samples drawn were used for estimation of electrical conductivity, pH and soil Nitrogen content. Soil pH was measured at 1:1.25 soil to water ratio using pH meter

with calomel glass electrode assembly. Electrical conductivity of soil sample after addition of 100 ml distilled water to 1 g soil samples was measured by conductivity meter. Total nitrogen content in soil samples was determined by the method of micro-Kjeldahl method [Jackson, 1973].

The pot experiment was laid in complete randomized design (CRD) with six treatments (Rec. N, absolute control (0 N), Nanofertilizer @ 25, 50, 75 and 100 kg N eq) and four repetitions each. For optimal growth, paddy needs 100:50:50 kg N, P₂O₅, K₂O ha⁻¹ N, as recommended in package of practises by UAS, Bengaluru, Karnataka, corresponding to 543 mg N, 0.78 mg P and 208 mg K per pot. The pot with complete recommended dose of fertilizer was kept as positive control and pot with no nitrogen supply was kept as negative control.

Planting material

Aerobic rice was selected as experimental crop mainly because paddy is staple food and is consuming major portion of fertilizers. Of late, due to scarcity of water resources, paddy cultivation is facing hindrances and farmers are looking for alternative methods like aerobic rice. Application of nitrogenous fertilizers faces greater losses under non water logged conditions due to denitrification. To address this issue, aerobic rice was selected as an experimental crop. Paddy seeds of variety BI 33 were collected from aerobic rice lab, Advanced Centre for Plant Biotechnology, UAS, GKVK, Bengaluru and KMP seeds were collected from AICRP on Paddy, Zonal Agricultural Research Station, V.C. Farm, Mandya.

Sowing

BI 33 was sown in February 2017 in pots at the rate of two seeds per pot. KMP175 was sown during November 2017. Calculated

fertilizer according to the treatments was mixed with one kg soil was filled to the pots, after fertiliser application sowing was taken. The pots were then equally watered and were exposed to greenhouse sunlight.

Measurement of plant growth and yield parameters and data analysis

Following germination, plants were thinned to one plant per pot. Plant height, numbers of leaves, number of tillers, flag leaf length, panicle weight, panicle length, chlorophyll content, number of seeds per plant, total number of seeds, seed weight and Biomass weight was recorded adopting standard procedure. Data were analyzed statistically through one-way ANOVA in a general linear model.

Nitrogen release pattern

To study the nitrogen release pattern, an experiment was designed according to Manikandan and Subramanian [2014]. Small pots were filled with soil homogenised with urea and UHA as treatments.

The 100 ml of deionised water added from 1 to 40 days. Solutions were collected to determine nitrogen content in the water.

Results and Discussion

Synthesis and characterisation of the urea-coated hydroxyapatite nanoparticles

Hydroxyapatite and urea coated hydroxyapatite nanoparticles were analyzed using DLS, PXRD, SEM and FTIR. Per cent of calcium and phosphorous in hydroxyapatite nanoparticles was found to be 35% and 11% following energy dispersive X-ray spectroscopy. Urea coated hydroxyapatite nanoparticle showed good stability in the solution and recorded 135mV zeta potential as indicated by dynamic light scattering. The PXRD pattern obtained was similar to the

powder diffraction file for HA (ICDD PDF No. 09-0432) and urea coated nanoparticles showed similarity to the peaks obtained by Kottegoda *et al.*, [2011]. Peak corresponding to urea was observed in urea-hydroxyapatite PXRD pattern (Fig. 1). SEM images of HA particles indicated oblong rice grain shaped morphology with size range between 25-55 nm. Upon coating with urea the morphological shape remained same with size varying from 45-65 nm (Fig. 2 and 3).

FTIR spectrum obtained describes hydroxyapatite nanoparticles and urea modification of HA nanoparticles. The sharp intense narrow band @1030cm⁻¹ correspond to symmetric stretching mode of [PO₄³⁻]_{v₃} ions in hydroxyapatite. Broadened [OH] stretching absorption was observed @3372cm⁻¹ and 632cm⁻¹ indicating polymeric association of hydrogen bonding. Narrow double peak @605 cm⁻¹ indicates asymmetric and symmetric deformation modes of [PO₄³⁻]_{v₄} group (P-O-P). An absorption band of weak intensity @2177 cm⁻¹ is connected with combinations of [PO₄³⁻]_{v₃}, _{v₁} modes. These functional groups correspond to non-stoichiometric apatite HAp structure with a low crystallinity degree. Bonding environment of urea coated HA nanoparticles with HA is indicated by change in the peaks position. N-H stretching frequency is observed as doublet @ 3437 cm⁻¹ and 3329 cm⁻¹ with significant broadened peak

indicating asymmetric and symmetric N-H stretching respectively. Moderate intensity broad peak @ 1615 cm⁻¹ indicates strong N-H bending absorptions of primary and secondary amines. Weak narrow peak @1674 cm⁻¹ correspond to carbonyl group and medium intensity broadened peak @1461 cm⁻¹ indicate N-C-N stretching in urea. The functional groups observed in FTIR spectra indicate physical adsorption of urea onto hydroxyapatite nanoparticles (Fig. 4).

Nitrogen content

Urea coated HA nanoparticles were analysed for N content twice and found that the material contain 36 per cent N as against 46 per cent in conventional urea fertilizer. Kottegoda *et al.*, (2011) reported 33.2 % N content in the urea surface-modified nanoparticles, while Subbaiya *et al.*, (2012) reported 35% N in nanourea modified hydroxyapatite nanoparticles. In another study, Manikandan and Subramanian (2014) reported 28% N in nanoporous zeolite based urea fertilizer.

Effect of nano-fertilizer on the growth and development of paddy

Soil parameters

The properties of the soil used for study was evaluated and is presented in the table below:

	N (%)	pH	EC (dS m ⁻¹)
Season 1	0.013	7.8	0.41
Season 2	0.015	8.7	0.32

The soil used during the first season has medium nitrogen content (0.013% equivalent to 291 kg/ha) and neutral to saline soil reaction and normal conductivity, while soil of the second season showed alkaline soil reaction and medium nutrient content (0.015% equivalent to 336 kg/ha).

Observations on plant growth and yield parameters

Several studies have reported beneficial effect of nanofertilizer on plant growth. Nano-fertilizer application in rice that promoted the growth, development, total phenolic content

and antioxidant properties demonstrating the potential to improve crop production and plant nutrition [Benzon *et al.*, 2015]. The pot experiment was conducted for two consecutive seasons on the aerobic rice varieties BI 33 and KMP 175 to evaluate the effect of urea coated hydroxyapatite nanofertilizer and the results were compared with the recommended dose of conventional neem coated urea. The results clearly indicated that the newly synthesized nano-

fertilizer showed on par results on growth and yield parameters of aerobic rice varieties compared with conventional urea. In BI33, the yield attributes viz., panicle weight, number of seeds per panicle, plant height, number of leaves, number of tillers were significantly superior with nanofertilizer at 50% or 75% N equivalent compared to the control (N0), but was on par with 100%N through conventional fertilizer.

Table.1 EDS profile of hydroxyapatite and urea-coated hydroxyapatite nanoparticle

Element (%)	Hydroxyapatite	Urea-modified Hydroxyapatite
N	-	36.5
Ca	35.43	12.6
P	11.94	5.4

Table.2 Effect of urea coated hydroxyapatite nanofertilizer on yield and yield attributes of BI-33, aerobic rice

Treatment	Panicle length (cm)	Panicle weight (g)	No. of seeds per paicle	Seed weight (g)	Plant height (cm)	Number of leaves	Number of tillers	Number of filled grains
N0:50P:50K	12.85	3.70	203.00	13.57	84.58	22.83	10.33	180.33
N100:50P:50K	15.19	6.26	285.33	16.04	89.50	25.67	12.17	292.00
NF25:50P:50K	15.14	5.29	243.00	14.81	87.06	22.11	11.39	216.00
NF50:50P:50K	15.01	5.12	281.00	15.76	89.19	32.50	12.00	242.00
NF75:50P:50K	14.78	5.53	295.67	15.53	90.28	23.78	11.44	267.67
N100:50P:50K	15.07	4.80	222.33	14.76	85.90	23.33	11.33	201.33
S.Em.±	0.62	0.31	20.42	0.76	3.38	1.99	0.82	10.05
CD (p=0.05)	1.87	0.93	61.93	2.31	10.24	6.04	2.49	30.49
CV (%)	4.28	6.76	8.69	5.24	3.91	8.25	7.29	4.79

Table.3 Effect of urea coated hydroxyapatite nanofertilizer on yield and yield attributes of KMP 175, aerobic rice

Treatment	Panicle length (cm)	Flag leaf length (cm)	Biomass weight (g/plant)	Seed weight (g)	Plant height (cm)	Number of leaves	SPAD value	Number of filled grains
N0:50P:50K	17.55	51.19	16.44	9.27	90.25	10.50	33.99	86.50
N100:50P:50K	17.69	53.06	17.38	15.12	92.00	13.00	34.85	111.61
NF25:50P:50K	18.10	50.88	16.25	12.29	94.50	13.50	34.51	103.05
NF50:50P:50K	18.41	53.73	14.88	14.99	91.38	12.00	34.23	114.11
NF75:50P:50K	18.65	53.44	12.79	14.36	95.63	11.25	34.29	112.39
N100:50P:50K	19.25	52.33	13.50	14.53	94.13	11.38	35.49	110.24
S.Em. _±	0.29	0.46	0.29	0.21	0.53	0.20	0.41	0.85
CD (p=0.05)	0.89	1.39	0.89	0.65	4.97	1.89	1.23	2.57
CV (%)	12.80	11.85	14.06	10.92	10.31	10.94	12.90	15.35

Fig.1 Powder X-ray diffraction pattern of (a) synthesized hydroxyapatite nanoparticles (b) urea coated hydroxyapatite nanoparticles

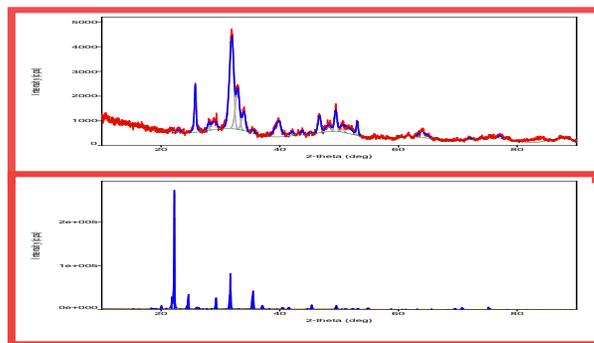


Fig.2 Scanning Electron microscopic image of (a) synthesized hydroxyapatite nanoparticles (b) urea coated hydroxyapatite nanoparticles

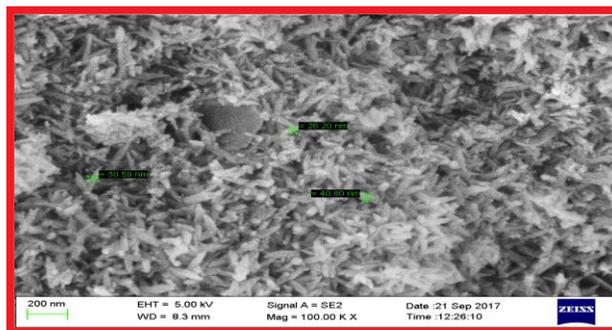


Fig.3 Particle size distribution of synthesized hydroxyapatite nanoparticle

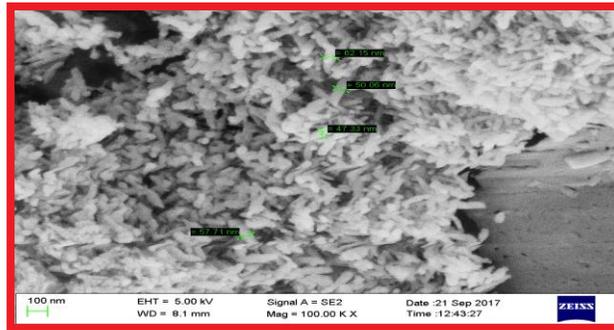


Fig.4 Fourier transform infrared spectra of (a) synthesised hydroxyapatite nanoparticles (b) urea coated hydroxyapatite nanoparticles

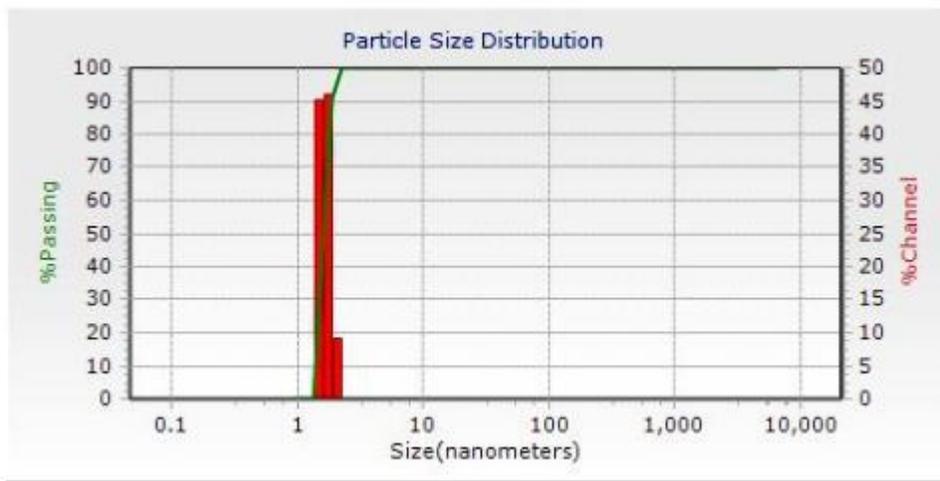


Fig.5 Effect of urea coated hydroxyapatite nanofertilizer on seed weight of aerobic rice varieties

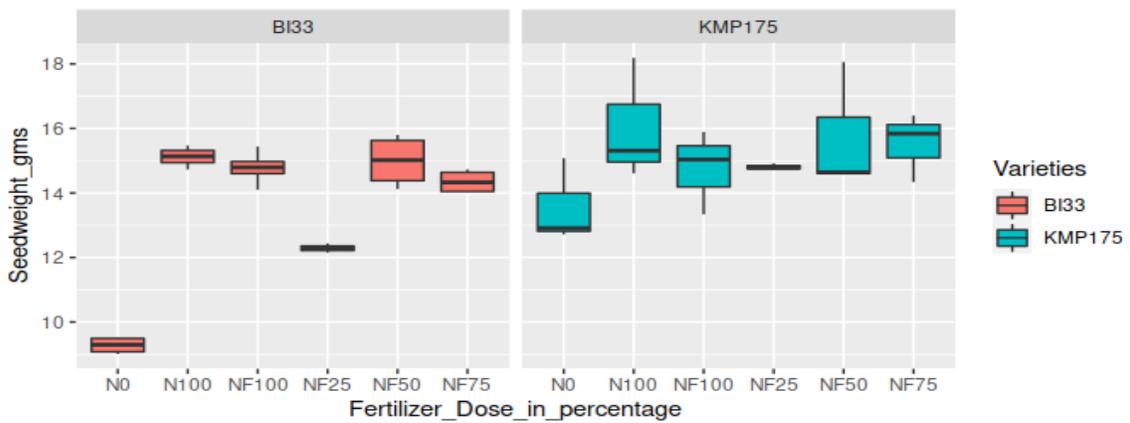
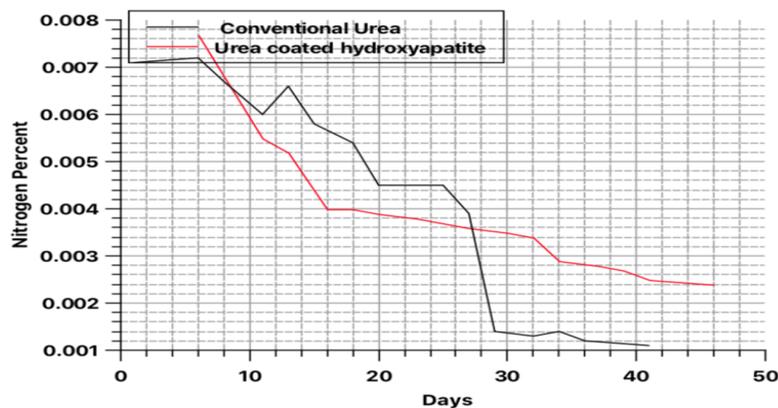


Fig.6 Elution profile of conventional urea and urea-coated hydroxyapatite nanoparticle

The seed weight was higher with 100% conventional fertilizer (16.04 g), but was statistically at par with different levels of nanofertilizer. Among the nanofertilizers, 50%N equivalent showed higher seed weight (15.76 g/plant) followed by 75%N (15.33 g/plant). In KMP 175, panicle length, flag leaf length, plant height and number of filled grains per panicle was significantly higher with nanofertilizer with 50 and 75% N equivalent compared to control (N₀). However, it was on par with 100%N through conventional fertilizer. Although, higher values of biomass weight, seed weight, and number of leaves were recorded with recommended conventional 100%N fertilizer, it was at par with nanofertilizers at different levels.

The superiority in the yield parameters with nanofertilizers is associated with betterment of growth parameters viz., plant height, chlorophyll indicated with SPAD. Similar results of nanonutrition in rice were noticed by Benzon *et al.*, [2015]. The enhanced growth in aerobic rice with 50%N and 75%N at par with 100% conventional fertilizer is associated with better nutrient availability and uptake leading to improved nitrogen use efficiency. These suggest that nanofertilizer can either provide nutrients for the plant or

aid in the transport or absorption of available nutrients resulting in better crop growth. Related study by Liu and Lal [2015] revealed similar findings in soybean. Nitrogen being a component in chlorophyll, efficient nitrogen utilization with lower dose of nanofertilizer improved the photosynthetic rate and assimilation resulting in better growth and yield attributes, These results are in harmony with those reported by Benzon *et al.*, [2015]; Jyothi and Hebsur [2017].

However, UHA nano-fertilizer at 50%, 75%, 100% dosage did not show significant differences in growth and yield, in fact plants performance decreased relatively at 100% dosage. This might be associated with the saturation of nitrogen demand of rice at 75%N with nanofertilizer formulation. Further, increased n levels followed Mitscherlich yield equation. The results are presented in Table 1, 2 and 3.

Nitrogen release pattern

Nitrogen release was quicker from soil containing conventional urea while UHA containing soil showed gradual release which indicates that the synthesized nanofertilizer releases the nutrient in step-by-step manner.

In conclusion the nanofertilizer composed of < 90 nm size urea-coated hydroxyapatite nanoparticles with 36% nitrogen was synthesized and was characterized using DLS, XRD, SEM and FTIR. The synthesized nanofertilizer showed equivalent performance to 100% conventional urea. Therefore, the current fertilizer dosage recommended for aerobic rice varieties can be reduced without compromising yield. However, the further studies are required to confirm the results under field conditions. The study indicates the improvement of fertilizer use efficiency by reducing nitrogen losses in an eco-friendly manner and to reduce environmental pollution.

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Declaration of interest statement

There is no conflict of interest exists among the authors.

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