Asian Journal of Chemistry; Vol. 33, No. 1 (2021), 159-165

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.22975

Effects of Formulated Nano-Urea Hydroxyapatite Slow Release Fertilizer Composite on the Physical, Chemical Properties, Growth and Yield of *Cyamopsis tetragonoloba* (Cluster Beans)

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Received: 31 August 2020; *Accepted*: 17 October 2020; *Published online*: 10 December 2020; AJC-20192

Urea and phosphorous fertilizers are commonly used in agriculture but, due to their solubility in water and transportation, cause eutrophication. Hence, it is thought worthwhile to investigate for urea hydroxyapatite nanoparticles which have less mobility and could supply required N and P macronutrients to the crops. These high surface area nanoparticles are synthesized through chemical co-precipitation method and it is assumed that due to their biocompatibility, act as rich phosphorous and nitrogen source. These are characterized by powder X-ray diffraction (PXRD), dynamic light scattering (DLS), scanning electron microscope (SEM), energy dispersive X-ray analysis (EDX) and Fourier transform infrared (FT-IR). The impact of urea hydroxyapatite nanofertilizer on growth and yield of cluster bean plants for the period of four months has been carried out. The experimental results have shown that the usage of these nanofertilizers have enhanced both the plant growth and yield. The application of urea hydroxyapatite nanocomposites for the bio-availability of plants considered to be environment friendly.

Keywords: Macronutrients, Urea hydroxyapatite, Nanofertilizers, Bioavailability, *Cyamopsis tetragonoloba*.

INTRODUCTION

The rapid growth of human population demands for increasing the agricultural resources throughout the world. The beginning of Green revolution during 1970′s solved the problem of hunger in many developing countries [1]. According to the World Food Organization (FAO), usage of chemical fertilizers is the most important factor in increasing global agricultural production in past three decades [2]. The main reason to use fertilizers is to give full fledged macro and micro nutrients for plants, which usually soil lacks. Around 35 to 40% of crop productivity depends mainly upon fertilizers. The elements N, P, K are considered to be essential macronutrient fertilizers, as N promotes leaf growth, forms proteins and chlorophyll, phosphorus contributes to root, flower and fruit development, potassium contributes to stem, root growth and also synthesis of protein [1,2]. Total global macronutrient fertilizers ($N+P_2O_5+K_2O$) consumption was 175.7 million tons in 2011 and was projected to increase to 263 million tons till 2050 [3]. It was estimated that nitrogen fertilizer has contributed roughly 40% increase in percapita food production in the past 50 years indicating the critical role of these macronutrient fertilizers in global food production [4]. Generally nitrogen is supplied in the form of urea to fulfill the macronutrient availability to plants. Due to high solubility of nitrogen fertilizers and their potential vulnerability to leaching and denitrification [5] (especially in the form of nitrates), it is estimated that 40- 70% is lost to environment and cannot be absorbed by plants [6,7].

Next to nitrogen, phosphorus accounts for 80 to 90% of world demand [8]. It is supplied in the form of mono ammonium phosphate (MAP, $NH_3H_2PO_4$), diammonium phosphate (DAP, $(NH_3)_2HPO_4$), triple super phosphate (TSP, $Ca(H_2(PO_4)_2$). These Phosphorus fertilizers are highly soluble in water and easily transported through surface runoff causing eutrophication. It is estimated that 80-90% of phosphorus applied, are lost causing environmental pollution. In addition, heavy usage of these fertilizers results in significant loss, where by these fertilizers contaminate the surface and groundwater bodies disturbing aquatic ecosystem and human health [8]. Therefore, it is of great concern to develop environmental friendly, high productive macronutrient (N+P) nanofertilizers [9] in place of the conventional urea and phosphorus fertilizers.

Application of nanotechnology in the field of agriculture and food sectors is in the stage of emergence, when compared to its usage in medicinal and material sciences [10-12]. Administration of macronutrients in the form of nanofertilizers, polymer coated fertilizers, nano-coated fertilizers will reduce the rate of dissolution of nutrients and allow slow, sustainable release of fertilizers, and assumed to be more efficiently absorbed by plant root system [5]. Phosphate in the form of phosphate rock and apatite has less solubility but accessibility of phosphorus from reaching the root zone and nurturing of the crop is hindered due to its larger particle size [13]. To minimize this problem, a new class of phosphorus fertilizer nano-hydroxyapatite was synthesized and reported earlier to enhance the growth and yield of soya beans through greenhouse study [14]. This nano-hydroxyapatite has the property of low solubility and long-term availability to plant system, which is due to increased surface to volume ratio of nanoparticles. Thus nano-hydroxyapatite acts as a good source of phosphorus in enhancing the agronomic yield and also reduces the risk of eutrophication.

Few attempts were made earlier to reduce the leaching of nitrogen into soil and to enhance the nutrient utilization efficiency by synthesis of urea hydroxyapatite nanohybrid. It was reported that the synthesis of nanohybrid of urea hydroxyapatite at weight percentage ratio of 1:1 that was incorporated into wooden chips and confirmed to achieve slow release of urea [15]. Though nanohybrid of urea hydroxyapatite synthesized by flash drying process, which is relatively cumbersome [16].

In the present study, a sincere attempt is made to synthesize the nanohybrids of urea hydroxyapatite through a simple, versatile and economical route of wet chemical co-precipitation method. Chemical precipitation of nano-sized fertilizers from salt solution is a simplest technique for rapid synthesis of large amount of material in a controlled manner [17]. This synthesis needs qualified and controlled parameters such as pH, composition of starting material, rate of addition, stirring speed, stirring technique and aging to obtain nanohybrids of urea hydroxyapatite. Urea hydroxyapatite nanohybrids are generally employed in agriculture because of high nutrient utilization efficiency, slow release of nitrogen and source of phosphorous macronutrients. Apart from that it is also useful to minimize adverse effects on environment.

Synthesized urea hydroxyapatite nanocomposites are characterized by XRD, SEM, EDX, particle size analyzer (DLS), FT-IR to confirm size of the crystallite, morphology, elemental composition and the functional groups of the sample. Therefore, the confirmed nanohybrid of urea hydroxyapatite are used to study the germination, growth and yield of cluster beans plants and to investigate the effect of bioavailability of nitrogen and phosphorus over conventional urea and diammonium phosphate (DAP).

EXPERIMENTAL

Synthesis of nanohybrid of urea hydroxyapatite: All the glassware used for the synthesis of nanoparticles were washed thoroughly with deionized water and dried in hot air oven before their use. Nanohybrid of urea hydroxyapatite sample was prepared by chemical coprecipitation method [16,18]. A definite amount of phosphoric acid (A.R. grade 99%, Finar) was dissolved in deionized water to form 0.6 M solution. A definite amount of calcium hydroxide (A.R. grade 96%, Finar) was also dissolved in deionized water to form 1M solution. To $Ca(OH)_2$ solution 150 g of urea (A.R. grade, Finar) was added. Then the solution was kept for constant stirring (exothermic reaction in nature). Now, 0.6 M H_3PO_4 solution was added dropwise to $Ca(OH)_2$ solution with constant stirring. After the completion of H_3PO_4 solution addition, the mixture was kept for constant stirring for 6 h at ambient temperature, a white curdy precipitate was obtained. It was kept for aging for 24 h. Thus, obtained product was filtrated using vacuum suction and washed several times to remove impurities. The product was dried in hot air oven at 90 ºC for 3 h. Finally, obtained product was grinded properly using agate motor pestle and stored for further studies.

Soil sampling: To study the germination and growth of cluster beans, soil sample (top soil of 8 inches depth) was collected from Vignana Bharathi Institute of Technology campus and analyzed. The physico-chemical characteristic parameters of soil [19] are shown in Table-1.

TABLE-1
COMPOSITION OF MACRONUTRIENTS IN THE TEST SOIL

Soil type	pH	N (Kg/Acre)	P (Kg/Acre)	K (Kg/Acre)
Red loamy soil	6.98	190.66	62	50.84

Seeds: Seeds of cluster beans were purchased from Indosun Agri Genetics, Hyderabad, India. The seeds were treated with 5% sodium hypochlorite solution for 10 min [20]. Then, washed with deionized water for 3-4 times to ensure surface sterility before sowing them in the soil.

Preparation of fertilizing solution: All the fertilizing solutions (urea hydroxyapatite (UHA), urea, diammonium phosphate) were prepared using deionized water with concentration of 20 ppm and ultrasonicated (ultrasonic cleaner model: BTI-48, 50 watts) for 30 min for uniform dispersion.

Pot studies: Germination and growth of cluster beans were conducted in four different batches, where each batch has three replicates (4×3=12 pots). Pots used for the study are of 12cm height (medium size). Each batch was supplied with 4 different solutions and the details are shown in Table-2.

TABLE-2
TREATMENT OF FERTILIZING SOLUTIONS PER BATCH

Batch	Pot labelling	Type of fertilizing solution applied
1	Urea hydroxyapatite	20 ppm of nano UHA solution
2	Conventional phosphorus	20 ppm of DAP solution
3	Conventional urea	20 ppm of urea solution
4	Control	Only deionized water

All the fertilizing solutions were used to assess the nitrogen and phosphorous uptake and also yield of cluster bean pods. Now six equal sized seeds of cluster beans per pot (6×12=72 seeds) were selected and soaked overnight separately in above prepared fertilizing solution. The soaked seeds are then sown into respective pots labeled as urea hydroxyapatite, Conv.P, Conv.Urea and control at a depth of 1.5 cm and covered with thin layer of soil in the first week of February 2019. Fertilizing solution was supplied for every 5 days to each batch. Besides, deionized water was supplied when needed, to ensure that only the nutrients present in soil and such supplied nutrients are available for the plant growth and yield.

Plant growth: The shoot length of the plants was measured for every 5 days. After 50 days, *i.e.* last week of March 2019 three plants per pot were removed to measure the root length. Thus removed plants were washed with deionized water for 3-4 times to remove soil and air dried to find fresh plant weight. After weighing the plants, they were dried at 70 ºC for 10 h to calculate dry weight.

Plant yield: The left over plants in each pot were allowed to grow further. The tiny cluster beans appeared in the second week of April 2019 and were harvested after a week. The fresh and dry weight of pods was calculated. The dry weight was obtained after drying at 80 ºC for 2 days in hot air oven.

RESULTS AND DISCUSSION

PXRD analysis: The sample was characterized by using xperto pro PHILIPS Powder X-ray Diffractometer with CuKα radiation = 1.5418 Å and 2θ ranging from 10-70º at 40 kV, 30 mA. The PXRD (Fig. 1a) peaks of UHA sample at 2θ values 21.95º, 25.61º, 29.03º, 31.64º, 39.79º, 46.54º, 49.37º, 52.95º, which have shown good concurrence with PXRD pattern of urea (JCPDS-89-2835) and hydroxyapatite (JCPDS-09-0432) [15].

Presence of XRD peak at 2θ value of 31.64º with plane of 211 corresponds to hydroxyapatite. In addition, another major peak at 21.95º with plane 110 indicates that the presence of urea. Reduction in the intensity of 21.95º peak in urea hydroxyapatite shows that such formed compound is amorphous due to the interaction between urea and hydroxyapatite. The average crystallite size was determined by Debye-Scherrer equation D=Kλ/(βcos θ) which was found to be 20 nm.

Morphology studies: The SEM micrograph of nanohybrids of urea hydroxyapatite recorded from Zeiss EVO [18]. The nanoparticles have shown strong agglomeration due to presence of hydroxyl group (Fig. 2). The particle size was found to be 1 µm and observed to be dispersed randomly.

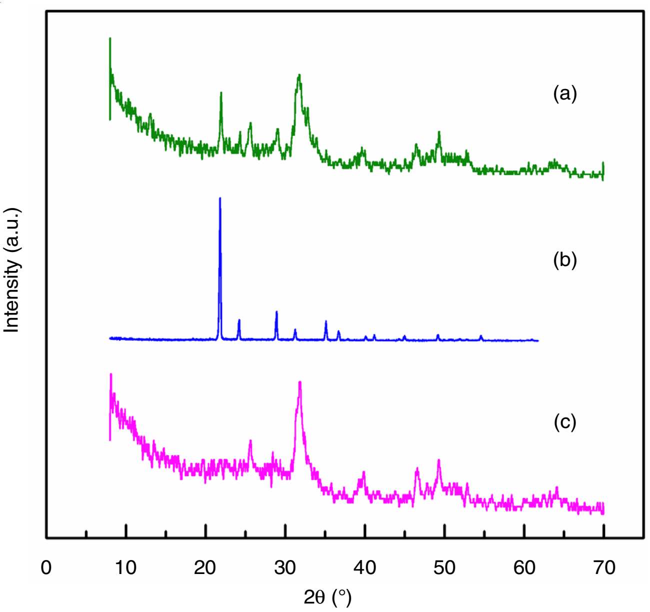

Fig. 1. PXRD pattern of (a) urea hydroxyapatite nanoparticles, (b) urea, and (c) hydroxyapatite

The EDAX spectrum of nanohybrids of urea hydroxyapatite (Fig. 3) also confirmed the co-existence of urea and hydroxyapatite. The spectrum (Table-3) showed good intensity peak of N, C, O, Ca and P.

TABLE-3
ELEMENTAL COMPOSITION OF UREA HYDROXYAPATITE

Compound	N (%)	C (%)	O (%)	Ca (%)	P (%)
Nano UHA	38.51	21.31	24.63	4.1	11.44

Particle size analyzer (DLS): Particle size of nanofertilizer urea hydroxyapatite was also analyzed with the help of Horiba SZ 100 particle size analyzer-Dynamic Light Scattering (DLS). The mean particle size of urea hydroxyapatite which was experimentally obtained to be 41.2 nm and interpreted to show the most probable polydispersity (Fig. 4).

FTIR analysis: The FT-IR spectrum of urea hydroxyapatite was recorded using JASCO IR-5300 spectrometer in the form of KBr pellets covering the wave number range 4000-400 cm^{-1}. The synthesized nano-urea hydroxyapatite was analyzed and confirmed by FT-IR spectrum (Fig. 5a-b). The bands obtained at 567 and 602.5 cm^{-1} correspond to the anti-symmetric

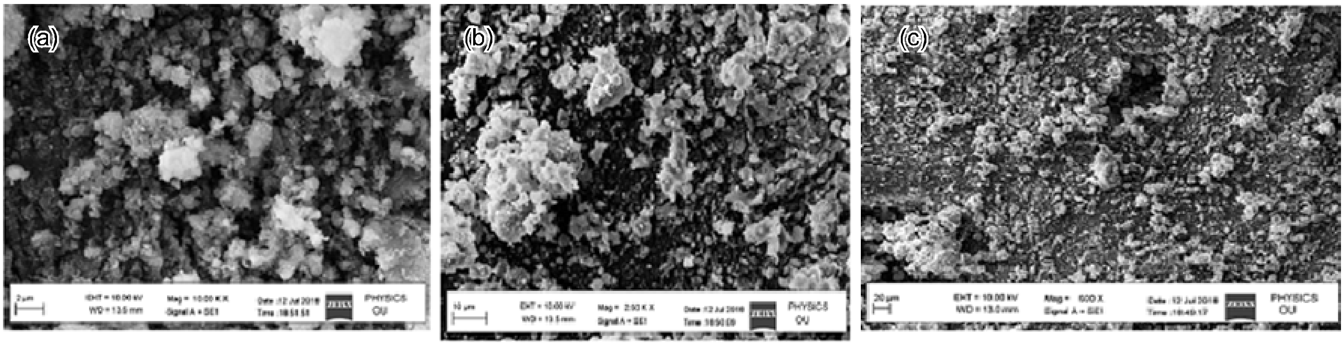

Fig. 2. SEM images of urea hydroxyapatite at (a) 2 µm, (b) 1 µm, (c) 20 µm

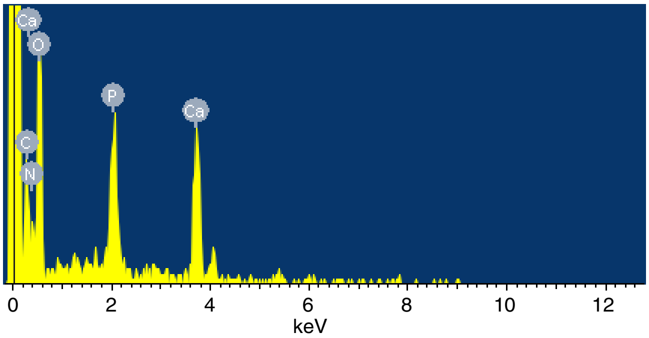

Fig. 3. EDX images of urea hydroxyapatite

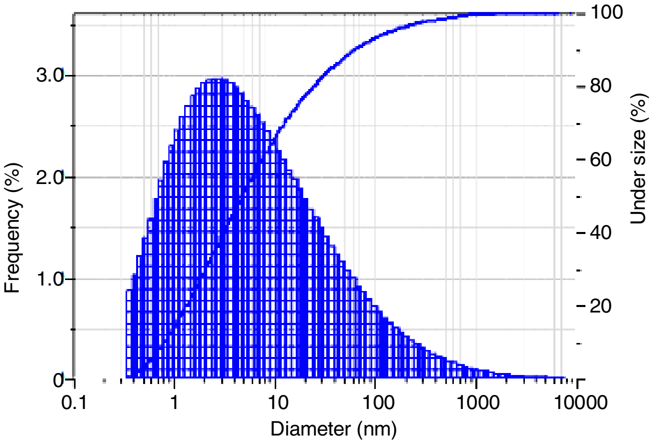

Fig. 4. DLS image of urea hydroxyapatite

bending of phosphate moiety in urea hydroxyapatite [17]. Similarly, the bands appeared at 1038.15 and 960 cm^{-1} are correlated to anti-symmetric and symmetric mode of stretching vibrations of P-O of PO_4^{3-}, respectively [21]. A broad band centered at 3368.3 cm^{-1} is attributed to OH group indicating the presence of water in the lattice of urea hydroxyapatite [22]. In addition, the CO and CO-NH wagging of pure urea is appeared at 793.12 cm^{-1}, which was shifted to 785.41 cm^{-1} in urea hydroxyapatite. A band at 1003 cm^{-1} representing the N-C-N symmetric stretching vibration in urea was shifted to 960.55 cm^{-1} in urea hydroxyapatite. A noticeable peak at 1466 cm^{-1} indicating the asymmetric stretching vibrations of N-C-N in urea which was shifted to lower wave number of 1454 cm^{-1} in urea hydroxyapatite. A band at 1151 cm^{-1} resembling to the rocking of NH_2 in urea was shifted to 1107 cm^{-1} in urea hydroxyapatite. The C=O stretching frequency of pure urea at 1672 cm^{-1} was changed to 1621 cm^{-1} in urea hydroxyapatite confirmed presence of the carbonyl group [23]. Thus FT-IR data reveals that urea was adsorbed randomly with hydroxyapatite through various binding sites.

Plant growth analysis: The synthesized nanofertilizer was used as a good source of nitrogen and phosphorous for the growth of cluster beans in present pot studies. The batch of nano-urea hydroxyapatite has shown good results in comparison with conventional urea, conventional phosphorus and control. Average shoot length of all batches was calculated for every 5 days. On 50th day after germination, urea hydroxyapatite treated plants have shown maximum shoot length of 15.5 cm when compared to plants treated with urea (11.4 cm) and diammonium phosphate (14.1 cm) (Fig. 6). Though diammonium phosphate treated plants have shown comparatively less growth than urea treated plants in first 25 days later it shooted up. The deionized water treated plants (control) have also shown considerable growth due to available nutrients in soil.

After 50 days of time period some plants from each batch were removed to calculate root length, plant fresh and dry mass. The average root length (Fig. 7) was found to be 8.2, 6.2, 6.4

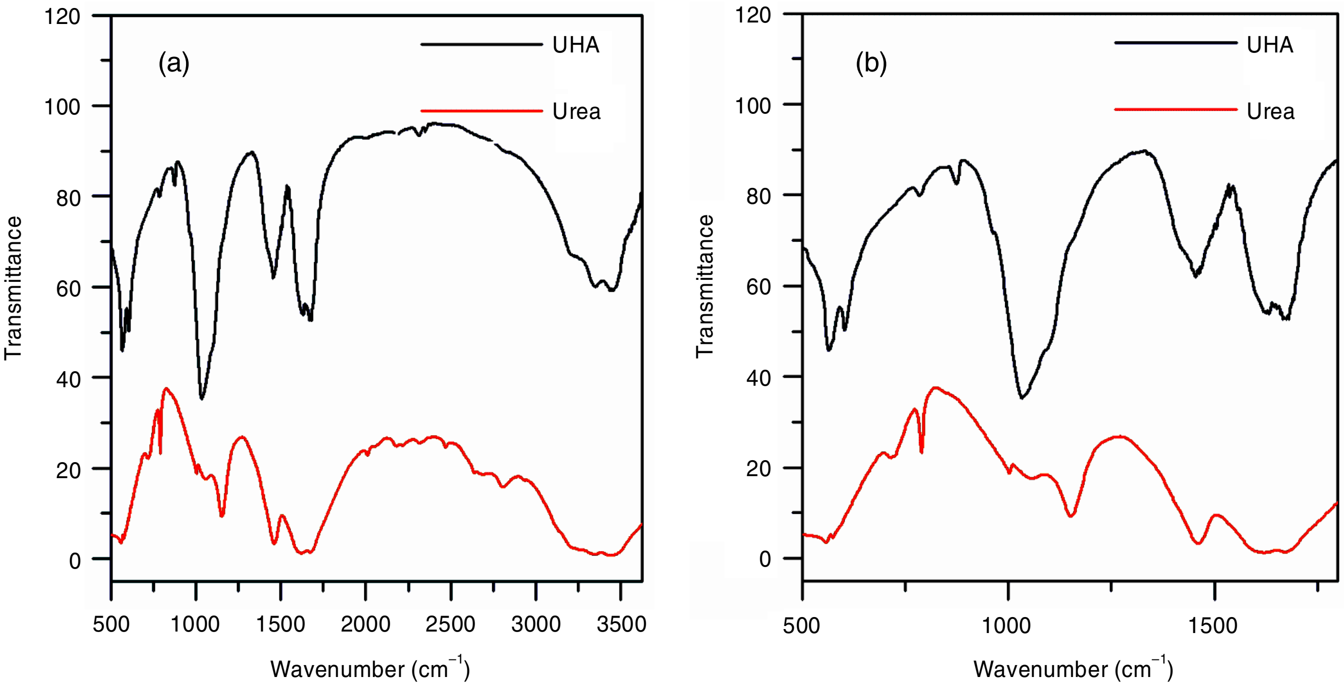

Fig. 5. FTIR spectrum of urea hydroxyapatite and urea at the wavenumber range from (a) 500 to 3500 cm^{-1} and (b) 500 to 1750 cm^{-1}

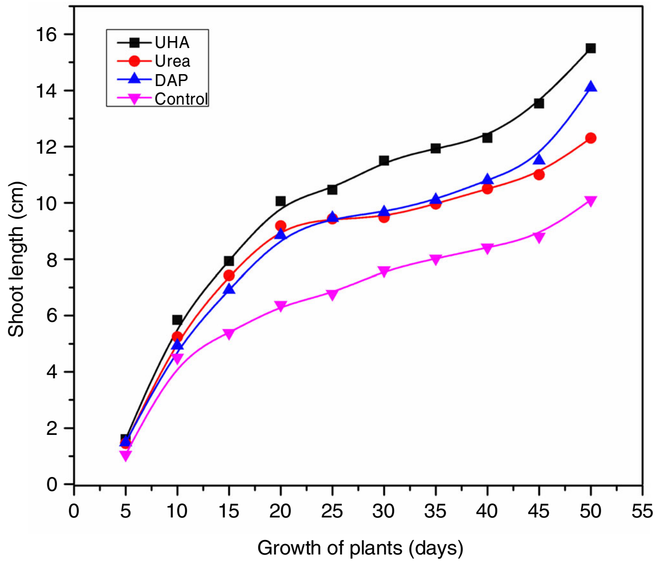

Fig. 6. Plot of plant growth analysis for 50 days

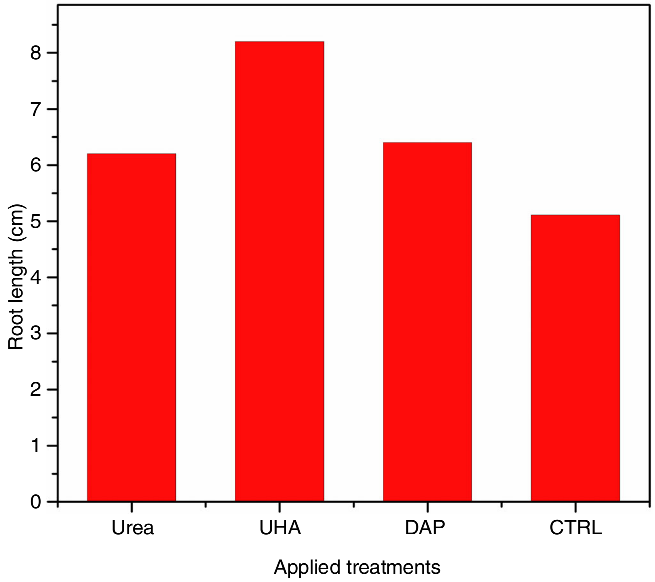

Fig. 7. Plot of root length under different fertilising solutions and control

and 5.1 cm for urea hydroxyapatite, conventional urea, conventional phosphorus and control, respectively. This indicates relatively good growth in tap root system of urea hydroxyapatite treated plants when compared to others.

The plant fresh weight (Fig. 8a) was found to be 1.71, 1.30, 1.35 and 0.26 g for urea hydroxyapatite, conventional urea, conventional phosphorus and control, respectively. The dry weight of plants (Fig. 8b) was also found to be greater for urea hydroxyapatite treated plants than others.

The first harvest of cluster beans of all batches was recorded and shown in Table-4.

TABLE-4
REPRESENTING THE PLANT YIELD

Batch	Pods fresh weight (g)	Pods dry weight (g)
Urea hydroxyapatite	82	48
Conventional phosphorus	64	39
Conventional urea	61	37
Control	50	26

By all these observations and from Fig. 9a-f, it was clear that urea hydroxyapatite treated plants have shown good nutrient utilization with respect to good growth of tap root system, shoot length, fresh weight, dry weight and yield. Thus, it was found that nanofertilizer urea hydroxyapatite can act as a good chemical fertilizer. Though cluster bean plant belongs to leguminaceace family that can fix nitrogen symbiotically, the results suggests the clear essentiality of N and P for their growth and yield.

Microscopic analysis: The transverse section of urea hydroxyapatite treated plant root (Fig. 10) when observed under compound microscope, it is clearly shown the layers of root cortex, endoderm, primary xylem and primary phloem (vascular bundles). The experimental facts further reveal the biocompatibility of nanofertilizer urea hydroxyapatite on cluster bean plants.

Conclusion

A simple and novel chemical co-precipitation method was reported for the successful synthesis of nano-urea hydroxyapatite, slow release fertilizer composite. The XRD study confirmed

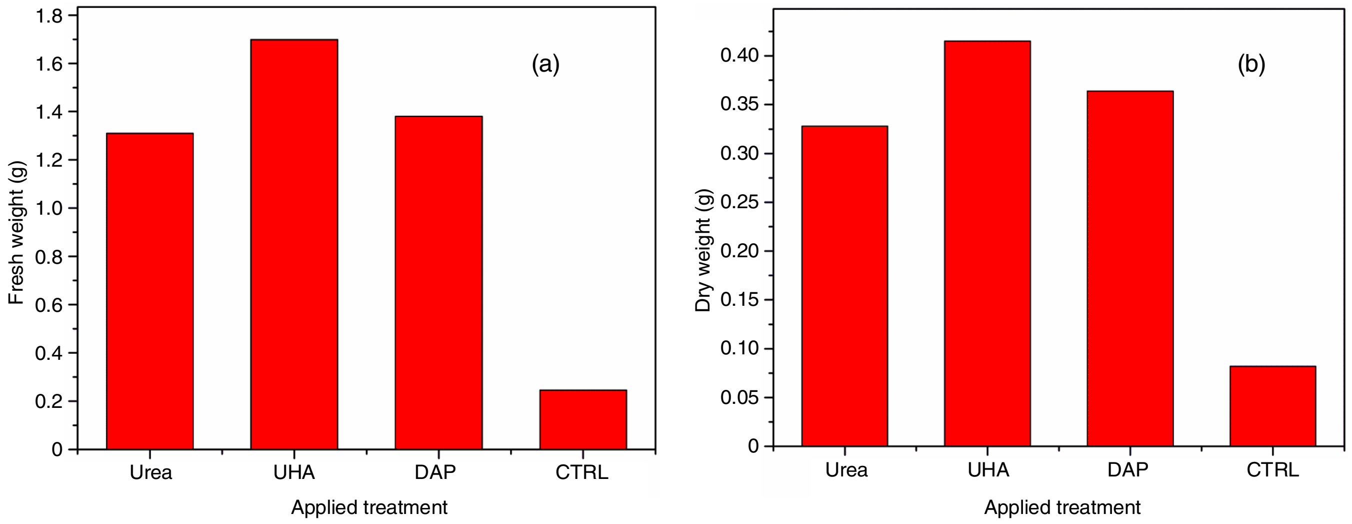

Fig. 8. Plot of plant fresh weight (a) and dry weight (b) under varied treatments

(a) Seeds started to germinate (b) Seedlings after 10 days (c) Seedlings after 1 month

(d) Seedlings after one and half month (e) Seedlings after 2 months (f) Seedlings after 2 months 10 days

Fig. 9. Showing growth of plants under treated fertilizing solution

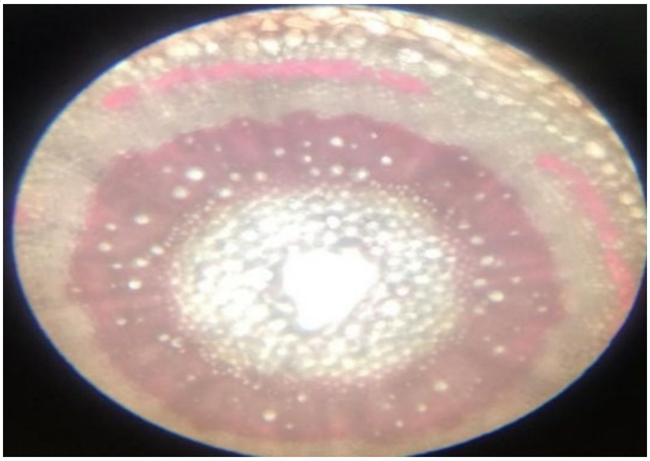

Fig. 10. Transverse section of urea hydroxyapatite root under compound microscope

the crystallite size as 20 nm. DLS also confirmed the particle size of urea hydroxyapatite. The SEM images showed that the particles are aggregated to form agglomerates. The EDX confirmed the existence of C, N, O, Ca, P stating the successful formation of urea hydroxyapatite. The pot studies of cluster bean plants over a period of four months showed that the application of urea hydroxyapatite, as a good nutrient source of nitrogen and phosphorous which has enhanced the growth rate (shoot and root length), plant biomass (fresh and dry weight) and also plant yield over that of conventional urea and diammonium phosphate. Microscopic studies of root of urea hydroxyapatite confirmed that there was no alteration in internal cell structure of the root, which further confirmed no negative impact on plant internal structure.

ACKNOWLEDGEMENTS

The authors acknowledge UGC, New Delhi for awarding the minor research project (UGC MRP/SERO ID: 6948/16). This project work has received constant encouragement and the authors are grateful to the Management of Vignana Bharathi Institute of Technology, Ghatakesar, Medchal, India.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. M.R. Khan and T.F. Rizvi, eds.: M. Ghorbanpour, M. Khanuja and A. Varma, Application of Nanofertilizer and Nanopesticides for Improvements in Crop Production and Protection, In: Nanoscience and Plant-Soil Systems, Springer International Publishing, Chap. 15, p. 405 (2017).
2. A. Mikhak, A. Sohrabi, M.Z. Kassaee and M. Feizian, *Ind. Crops Prod.*, **95**, 444 (2017); https://doi.org/10.1016/j.indcrop.2016.10.054
3. N. Alexandratos and J. Bruinsma, World Agriculture Towards 2030/2050: The 2012 Revision. ESA Working Paper No. 12-03, Rome, FAO (2012).
4. V. Smil, *J. Human Environment*, **31**, 126 (2002); https://doi.org/10.1579/0044-7447-31.2.126
5. J..S Duhan, R. Kumar, N. Kumar, P Kaur, K. Nehra and S. Duhan, *Biotechnol. Rep.*, **15**, 11 (2017); https://doi.org/10.1016/j.btre.2017.03.002
6. M.E. Trenkel Controlled-Release and Stabilized Fertilizer in Agriculture, In: International Fertilizer Industry Association Conference Proceedings, International Fertilizer Industry Association: Paris (1997).
7. A. Ombódi and M. Saigusa, *J. Plant Nutr.*, **23**, 1485 (2000); https://doi.org/10.1080/01904160009382116
8. D.L. Childers, J. Corman, M. Edwards and J.J. Elser, *Bioscience*, **61**, 117 (2011); https://doi.org/10.1525/bio.2011.61.2.6
9. R. Liu and R. Lal, *Sci. Total Environ.*, **514**, 131 (2015); https://doi.org/10.1016/j.scitotenv.2015.01.104
10. C.S. Chakra, K.V. Rao and V. Rajendar, *Dig. J. Nanomater. Biostruct.*, **12**, 185 (2017).
11. W.P.S.L. Wijesinghe, M.M.M.G.P.G. Mantilaka, R.M.G. Rajapakse, H.M.T.G.A. Pitawala, T.N. Premachandra, H.M.T.U. Herath, R.P.V.J. Rajapakse and K.G.U. Wijayantha, *RSC Adv.*, **7**, 24806 (2017); https://doi.org/10.1039/C7RA02166F
12. A.R. Kumar, K.V.G.R. Kumar, Ch. Shilpa Chakra and K.V. Rao, *Int. J. Emerg. Technol. Adv. Eng.*, **4**, 209 (2014).
13. N.K. Fageria, The Use of Nutrients in Crop Plants. CRC Press: Boca Raton FL, p. 430 (2009).

14. R. Liu and R. Lal, *Sci. Rep.*, **4**, 5686 (2015); https://doi.org/10.1038/srep05686
15. N. Kottegoda, I. Munaweera and N.M.A.V. Karunaratne, *Curr. Sci.*, **101**, 73 (2011).
16. N. Kottegoda, C. Sandaruwan, G. Priyadarshana, A. Siriwardhana, U.A. Rathnayake, D.M. Berugoda Arachchige, A.R. Kumarasinghe, D. Dahanayake, V. Karunaratne and G.A.J. Amaratunga, *ACS Nano*, **11**, 1214 (2017); https://doi.org/10.1021/acsnano.6b07781
17. A.B.H. Yoruç and Y. Koca, *Dig. J. Nanomater. Biostruct.*, **4**, 73 (2009).
18. M.P. Ferraz, F.J. Monteiro and C.M. Manuel, *J. Appl. Biomater. Biomech.*, **2**, 74 (2004).
19. G. Gunaratne, N. Kottegoda, N. Madusanka, I. Munaweera, C. Sandaruwan, W. Priyadarshana, A. Siriwardhana, B.A.D. Madhushanka, U.A. Rathnayake and V. Karunaratne, *Indian J. Agric. Sci.*, **86**, 494 (2016).
20. P. Mahajan, S.K. Dhoke and A.S. Khanna, *J. Nanotechnol.*, **69**, 6535 (2011); https://doi.org/10.1155/2011/696535
21. A. Costescu, I. Pasuk, F. Ungureanu, A. Dinischiotu, M. Costache, F. Huneau, S. Galaup, P. Le Coustumer and D. Predoi, *Dig. J. Nanomater. Biostruct.*, **5**, 989 (2010).
22. M.-G. Ma, *Int. J. Nanomedicine*, **7**, 1781 (2012); https://doi.org/10.2147/IJN.S29884
23. Z. Piasek and T. Urbanski, *Bull. L'academie Pol. Sci. Serle Sci. Chim.*, **10**, 113 (1962).