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Two new plant nutrient nanocomposites based on urea coated hydroxyapatite: Efficacy and plant uptake

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ABSTRACT

Macronutrient delivery to plants, particularly nitrogen, is problematic because of losses occurring during fertilization. Currently, nanotechnology is being considered as a solution to improving nutrient use efficiency. In this study, we report the synthesis and plant uptake of two plant nutrient nanocomposites based on urea coated hydroxyapatite (U-HA) and potassium encapsulated into (i) a nanoclay, montmorillonite (MMT) or (ii) cavities present in *Gliricidia sepium* stem resulting in a wood chip containing macronutrients. Soil leaching behaviour, efficacy and plant uptake of the nutrients were tested in a pot experiment using *Festuca arundinacea* during a period of 60 weeks. Two nanocomposites displayed slow release behaviour particularly for nitrogen, in soil leaching tests compared to the conventional formulations. Both nanoformulations displayed efficient plant nutrient uptake highlighting the improved nutrient use efficiency. These data clearly revealed that urea fabricated into its nanoscale provide platform for development of efficient fertilizer formulations.

Key words: Encapsulation, Nanocomposites, Plant nutrients, Slow release,
Urea coated hydroxyapatite nanoparticles

Continued long term sustainable improvement in world food production is the key to achieving food and nutrition security in the future. Studies suggest that increases in world food production are feasible with better crop management and new technologies, particularly through increases in yields and reduction in production cost. In this context, fertilizer innovations and improved use efficiency play an integral role (Smil 2011). An adequate and efficient supply of plant macronutrients such as nitrogen (N), phosphorus (P) and potassium (K) are required if the genetic yield potential of crops are to be realized. The main problems relating to the use of N fertilizer in agriculture arise due to the use of fossil fuels in its production and the resulting emission of greenhouse gases during manufacturing process. The industrial conversion of nitrogen gas (N_2) into ammonia (NH_3) currently requires 4% of the world natural gas supply and 1-2% of the total energy production (Smil 2011). Therefore, considering the energy used in its synthesis and the large tonnage required, the cost of N fertilizer has a high monetary value. This is coupled to the loss of 50-70% of N applied using conventional fertilizers from soil due to leaching, low N utilization efficiency by plants, adding further to the cost of getting N into the plant, particularly in the tropical regions where the temperatures and soil moisture contents are relatively high. Leaching of N can happen by way of water soluble nitrates (NO_3^-), emission as NH_3 and nitrogen oxides (N_xO_y) and soil microorganism mediated incorporation into soil organic matter over time (DeRosa *et al*. 2010).

On the other hand, for P and K fertilizers, which are derived from mineral deposits, concerns are associated with limited supplies both geographically and quantitatively on a global scale. The issues of pollution of surface waters are pertinent to all N, P and K fertilizers.

Slow and controlled release fertilizers are believed to have the potential to address most of the fertilization challenges; although none of the available systems have shown a greater promise so far on a global scale (DeRosa *et al*. 2010). The emerging nanotechnology based strategies indicate that, due to their nanoscale size (1-100 nm) and high surface area to volume ratio (compared to macro sized conventional fertilizer systems), nanofertilizers would profoundly impact energy, economy, and environment by reducing nutrient loss due to leaching, emissions, and long-term incorporation by soil microorganisms (DeRosa *et al*. 2010, Karunaratne *et al*. 2012, Kottegoda *et al*. 2011).

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Nutrients encapsulated in nanoparticles may increase the uptake efficiency by triggering the release according to the environmental conditions and plant demand. In addition, slow and controlled release fertilizers may also improve soil by decreasing adverse effects associated with over application of fertilizer.

With the exception of a few investigations, there has been a paucity of research in examining the potential of nanomaterials, ranging from clays to other nanoparticles, to encapsulate plant nutrients and agrochemicals for slow release applications (Park *et al*. 2004, Torres-Dorante *et al*. 2009, Torres-Dorante *et al*. 2008, Wu and Liu 2008, Xiaoyu *et al*. 2013). On the other hand, the ability of hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH)_2$] nanoparticles in fertilizer applications had remained unexplored, except for the studies reported by (Kottegoda *et al*. 2011, Kottegoda *et al*. 2012).

In the reported study, HA nanoparticles, with its rich surface chemistry owing to the presence of reactive functional groups (Ferraz *et al*. 2007, Han and Misra 2009, Mateus *et al*. 2008, Mateus *et al*. 2007, Teng *et al*. 2009, Zhu *et al*. 2010) were explored for surface modification with urea.

MATERIALS AND METHODS

All reagents and chemicals used in this study were purchased from the Sigma Aldrich Company, USA and were of analytical grade and used without further purification. Sodium intercalated MMT (Na-MMT) clay was purchased from Southern Clay Ltd., USA. All solutions were prepared using distilled water. Analysis of data was carried out with the aid of Origin statistical package (version 8.0).

U-HA nanoparticles were synthesized as explained by Kottegoda *et al*. (2011). Liquid assisted grinding method was used to encapsulate U-HA nanocomposite into MMT clay matrix. U-HA nanoparticles (25 g) were mixed with Na-MMT (142 g) and ground in a grinder for 5 min. Water (25 ml) was added to the ground U-HA-MMT solid compound resulting in a paste and grinding was continued for further 20 min. The resulting composite appeared as wet granules after grinding and they were oven dried at 60 °C for 30 min.

In order to prepare K encapsulated MMT (K-MMT) potassium chloride (KCl) solution (100 g/l) was added drop wise into Na-MMT dispersion (66.67 g/l) in order to introduce K^+ to the nutrient composition through ion exchange reactions. The resulting composite was dried at 100 °C for 2 hr and the final product was obtained as clay flakes.

U-HA nanoparticles encapsulated wood chips (U-HA-wood) were prepared as explained by (Kottegoda *et al*. 2011). The resulting wood chips were dried at 60 °C for 2 hr and used for the soil leaching and plant uptake studies.

G. sepium stems (15.0 g) were cut into 0.5 × 0.5 × 0.5 cm^3 wood chips were soaked in KCl solution (230 g/l) and pressurized at 9 bar for 2 hr for the preparation of K impregnated wood chips (K-wood).The resulting wood chips were dried at 100 °C for 2 hr and used for soil leaching and plant uptake studies.

Table 1 Composition of different plant nutrient nanocomposites

Plant nutrient composites	N%	P_2O_5%	K_2O%	MgO%
HA nanoparticles		39.16		0.38
U-HA-MMT	8.44	7.29		0.70
K-MMT	0.58	0.99	12.32	0.44
U-HA-wood	20.18	2.41	1.35	0.37
K-wood	1.70	0.25	15.00	0.44
Conventional	11.00	11.00	11.00	

N, P and K amounts in the nanoplant nutrient systems prepared as explained above were evaluated using standard analytical methods and are summarized in Table 1.

The morphology of the U-HA nanoparticles and its encapsulated composites was studied using Hitachi SU 6600, Scanning Electron Microscopy (SEM).

Soil leaching and plant uptake studies were carried out using intentionally selected Ceylon tea soil obtained from 0-15 cm depth in the ground from high elevation, Sri Lanka (1 250 m above the sea level). Majority of the soils found in tropical regions like in Sri Lanka, are derived from fully weathered rocks. Therefore, these soils are rich in kaolinite which has very poor nutrient retention capacity and as a result fertilizers generally lose their efficiency within a short time period after application. The physical and chemical characteristics of soils are summarized in Table 2.

Soil (400 g) was mixed with conventional tea fertilizer mixture (N 11%, P_2O_5 11% and K_2O 11%) containing urea, rock phosphate and muriate of potash and packed into a column. Then, nanoplant nutrient compositions (weight adjusted to contain an equal amounts of NPK as the conventional fertilizer) mixed with the Ceylon tea soil were packed separately into two other columns. Water (180 ml) was added to all three columns in order to achieve water saturation point. Then, the nutrients in the soil were allowed to leach out by adding (100 ml) water in every four day intervals. The eluate (50 ml) was collected and the nutrient amounts were analyzed using standard analytical methods.

Moist soils were passed through a 6.4 mm sieve prior to starting plant uptake studies. Soils (4 kg) were mixed with different N, P and K amounts according to the standard recommendation for Ceylon tea, as explained (Wickramasinghe and Krisnapillai 2008). The amount of P (as P_2O_5) in U-HA-wood formulation was below the required amounts as per recommendations for Ceylon tea. In order to supplement the shortfall of P in the wood chip nanocomposite, HA nanoparticles were added separately as a P source.

Soils were filled into pots of 4.5 l capacity each holding

Table 2 Physico-chemical characteristics of soil

Soil location	Soil pH	Organic carbon (%)	N (%)	P (mg/ kg)	K (mg/ kg)	Mg (mg/ kg)
Ceylon tea soil	5.0	4.12	0.30	58	125	52

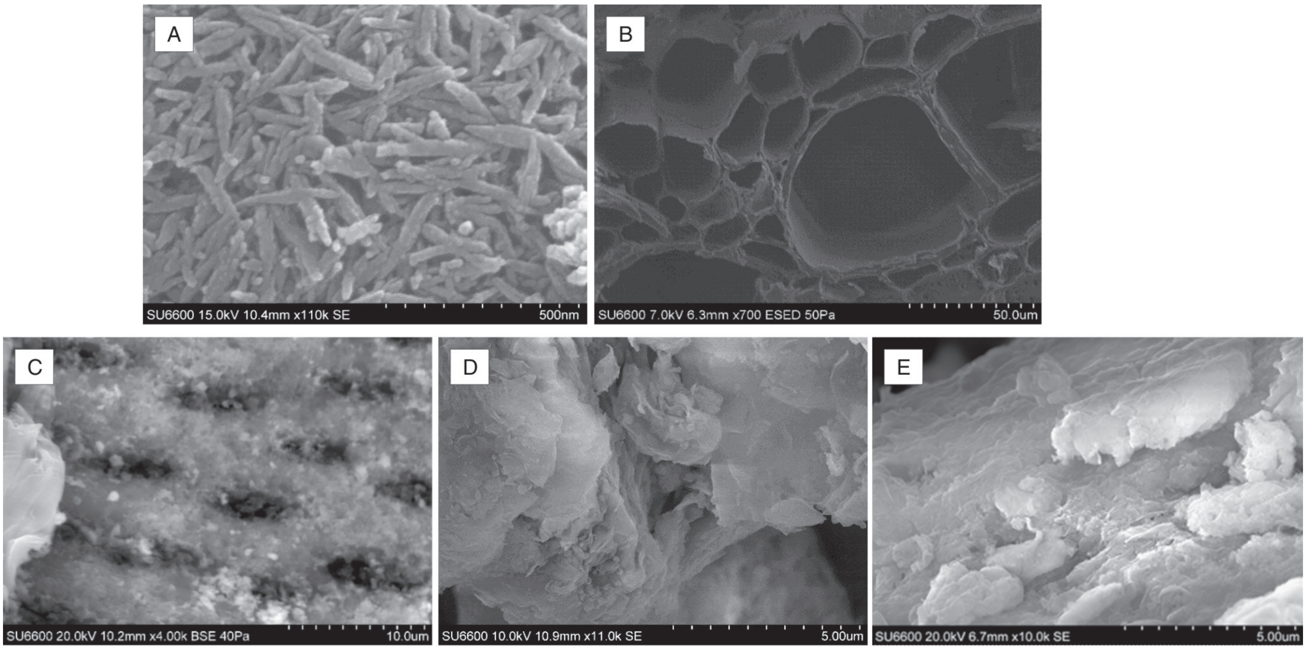

Fig 1 SEM images of: A, U-HA rod shaped nanoparticles; B, different sized cavities of G. *sepium* stem; C, after encapsulation of U-HA into *G. sepium* stem; D, MMT clay and E, U-HA-MMT nanocomposite.

Table 3 Treatments used for plant nutrient uptake efficiency studies

Treatment	Plant nutrient composition	Nutrient (g/pot)		
		N	P_2O_5	K_2O
T1	Conventional Ceylon tea fertilizer urea + rock phosphate + muriate of potash (*standard dose*)	1.8	1.2	1.2
T2	HA nanoparticles + U-HA-wood + K-wood (*50% of the standard dose*)	0.9	0.6	0.6
T3	HA nanoparticles + U-HA-wood + K-wood (*similar to standard dose*)	1.8	1.2	1.2
T4	U-HA-MMT + K-MMT (*50% of the standard dose*)	0.9	0.6	0.6
T5	U-HA-MMT + K-MMT (*similar to the standard dose*)	1.8	1.2	1.2
T6	Control (no fertilizer)			

4 kg of soil mixed with different nutrient formulations as explained in T1 – T6 (Table 3), separately.

Then, all pots were sown with 1.0 g of *F. arundinacea* seeds. Pots with plants were assigned to 12 completely randomized replicates, each replicate having 6 pots representing each treatment T1 – T6 as given above. Moisture content in soil was maintained at 60% by adding distilled water as and when required.

Grass was harvested from all pots at 4 to 8 week intervals starting from the 4th week after emergence, leaving 2.5 cm of stubble up to 60 weeks. These samples were dried in an oven at 80 °C and ground in a Willey mill resulting in 0.2 mm particles for N, P, K and Mg analysis using standard analytical methods.

The shoots resulting in each harvest were dried in an oven at 80 °C until constant dry weights are received and dry weights were recorded. During each harvesting the declining numbers of pots were noted for statistical analysis purposes.

The available N, P, K and Mg content remaining in the soil were analyzed at the end of the experimental period using standard methods. The leaves and roots of the plants were investigated under SEM (Hitachi SU 6600, secondary electron mode) to investigate the presence of nanoparticles within the plants.

Pot trial data was subjected to one way ANOVA with 99.5% confidence level for a randomized complete block design taking the decline in number of replicates into account as the experiment progressed. At each stage, data obtained from the destructive sampling was analyzed as for a randomized block design with 3 replicates.

RESULTS AND DISCUSSION

The successful synthesis and surface modification of HA nanoparticles with urea were confirmed by a number of characterization techniques (Kottegoda *et al.* 2011). The morphological characteristics of the resulting nanocomposites are shown in Fig 1.

Soil leaching tests over a period of 60 days provided preliminary evidence of the potential of the nanocomposites as novel slow release formulations, particularly for N, compared to the conventional fertilizer mixture, which is the hardest to retain in soil among the three macronutrients (Fig 2).

N uptake pattern of the treatments are shown in Fig 3A

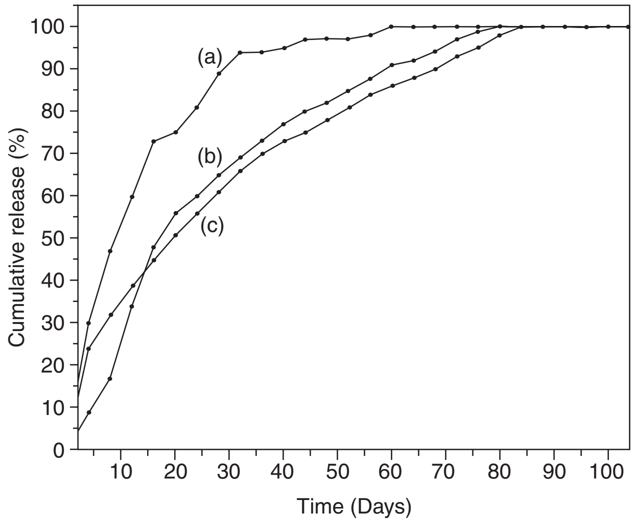

Fig 2 Cumulative release of N in soil: (a) urea, (b) U-HA-MMT, (c) U-HA-wood.

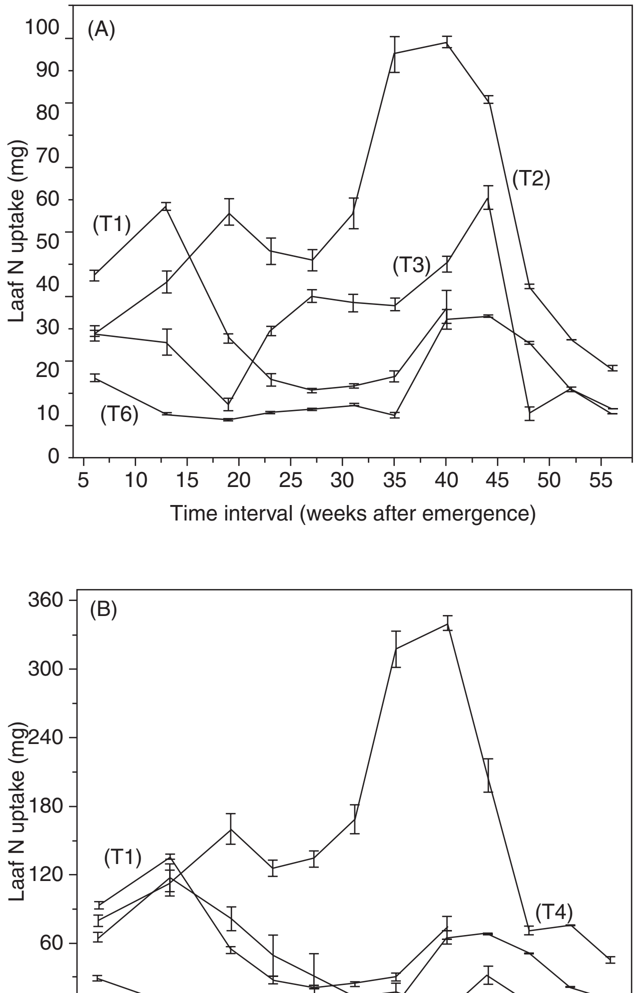

Fig 3 A and B: Leaf N uptake pattern of the treatments.

and B. At the beginning of the experiments, tea soil with no added fertilizer had relatively high nutrient content which was depleted to low levels at the end of the experimental period.

According to Fig 3A and 3B, a burst release of N was evidenced with the treatments containing the conventional fertilizer mixture amounting to the expected rate of loss of N through leaching and evaporation in the high elevation Ceylon tea soils in Sri Lanka. The rapid release of N in the treatments with the conventional plant nutrient system within the first 8 weeks after emergence is clearly observed in the N uptake patterns leading to an initial fast growth followed by a nutrient deficient condition in soil resulting in growth retardancy at the end of 40th week. This situation was clearly evidenced by the death of plants due to deficiency of nutrients while healthy plants were observed up to 60th week when nanoplant nutrient formulations were used.

Particularly, a continued leaf N uptake was observed in T2 and T4 when plant nutrient nanocomposites were used at half the recommended quantities within the period of study. Since, the N uptake pattern can be directly correlated to the availability of nutrients in soil, the observations suggest that both nanocomposites based on *G. sepium* and MMT have demonstrated slow release behaviour corroborating the results of the soil leaching experiments, indicating a synchrony between the plant N demand and uptake. For example, there was a significant leaf N uptake ($P < 0.005$) even at the 9th cut at 44 weeks after emergence, compared to the treatments with conventional fertilizer system. The treatments with U-HA-MMT based plant nutrient system at 50% dosage showed a higher uptake in comparison to U-HA-wood at 50%. Surprisingly, at full dosage, the nutrients applied with the nanoplant nutrient compositions did not demonstrate a significant difference in bioavailability, leading to retardancy in growth, compared to that at 50% dosage in nano-form, suggesting that the presence of excess of slow release fertilizer in constrained soil provides no significant advantage to plants. In these treatments, toxic effects to the plants were observed qualitatively, possibly due to the long term presence of plant nutrients in excess within a limited space.

A slow and controlled release behavior of K was observed for both nanoplant nutrient systems when applied at the half recommended dose.

In highly weathered soils, predominantly rich in kaolinite, cation retention capacity is very low. As a result, the highly soluble K salts applied to such soils are lost due to leaching (Kolahchi and Jalali 2007). In contrast, K^+ ions within the clay layers or cavities in *G. sepium* stem have leached out in a controlled manner.

Interestingly, bioavailability of Mg^{2+} ions was elevated at 50% dose of applied nanoplant nutrient systems although neither of them contains sufficient amounts of available Mg.

Gradual increase in the amount of P uptake up to 40 weeks after emergence was evidenced particularly with the MMT based plant nutrient system. On the other hand, in the

Table 4 Nutrients remain in the soil at the end of the experimental period

Treatment	At the end of the study period			
	N%	P (mg/kg)	K (mg/kg)	Mg (mg/kg)
T1	0.27	29	83	27
T2	0.28	26	162	28
T3	0.27	36	65	27
T4	0.25	55	133	33
T5	0.24	26	82	34
T6	0.26	65	165	32

conventional system, absence of available P through slow release can be linked to limited solubility of the applied rock phosphate.

In evaluating the efficiency of a slow release plant nutrient composition, the dry matter yield is one of the important indicators of the nutrient uptake and availability. The significant differences in the shoot dry matter yield among the six different treatments were observed.

As expected, the treatment containing the conventional fertilizer mixture displayed a higher yield at the beginning until the 2nd cut due to the presence of sufficient nutrients. The maximum leaf dry matter yield was observed with the plant nutrient nanocomposites at 50% dose throughout the period of study, suggesting the efficacy of the nanoplant nutrient formulations as future slow release applications. Table 4 summarizes the amount of nutrient content remaining in soil after the end of the experimental period.

The mechanisms of loss of N in conventional fertilizers are known. It is envisaged that one of the main qualities of a slow release fertilizer is to minimize this loss. Therefore, the soil nutrient availability versus plant uptake of a slow release fertilizer would have better parity than a conventional fertilizer. Encapsulation of the U-HA nanoparticles in MMT matrix leads to the formation of a U-HA-MMT nanocomposite would reduce the solubility of urea due to the electrostatic interactions and ensuing steric effect. Once these hierarchical nanoplant nutrient compositions are incorporated into a soil system, it would display pH dependent slow release behavior as observed in many of the slow release applications based on MMT and anionic clays (Joshi *et al.* 2009; Park *et al.* 2004; Torres-Dorante *et al.* 2009). Furthermore, when urea molecules are hydrolyzed to NH_4^+ ions, MMT can readily adsorb such NH_4^+ in a similar mechanism that is explained by Torres-Dorante *et al.* (2009) in the use of layered double hydroxides to buffer nitrate exchange properties in soil.

On the other hand, controlled release behavior of N in U-HA-wood system can be interpreted by considering its structure. In the plant nutrient composition based on the wood chip, U-HA nanoparticles were encapsulated into the nano, sub-micro and micro cavities present in the soft wood stem, *G sepium*. These cavities become reservoirs for storage of U-HA nanoparticles. When U-HA-wood plant nutrient system is incorporated into a soil system, it will absorb moisture, initiating slow and controlled release of N into the soil as a result of diffusion and microbial degradation which facilitates the encapsulated nutrients to leach out as a function of time. Urea localized in large vascular canals would be released early during the release process while those encapsulated in relatively smaller cell cavities can be expected to be released at an intermediate stage; smaller volumes of intercellular spaces may release N at the final stages during slow release process.

Furthermore, previous studies suggest the existence of synergistic interactions between the two nutrients N and K, where K assists the crop response to applied N fertilizers (Milford and Johnston 2012). In slow release formulations, balance between the two nutrients can be maintained thus facilitating optimum nutrient uptake by plants. It appears that the two slow release nanoplant nutrient systems based on MMT and *G. sepium* wood chip, allow for maintaining a balance between the N and K, leading to continuous uptake of both N and K throughout the experimental period, thus minimizing losses due to various factors compared to the treatments with conventional fertilizer formulations. In addition, K plays a major role in the transport of water and nutrients throughout the xylem. The availability of optimum amounts of K^+ leads to maintaining the water content of the vacuoles (Milford and Johnston 2012). According to the N and K uptake patterns and plant dry matter yield in nanocomposite formulations, the presence of optimum amounts of available K^+ ions have assisted the plant to maintain a constant concentration of salts in water to sustain the osmotic concentrations leading to healthy growth. Comparing the P uptake patterns, it is clearly demonstrated that the available P for plant uptake is higher in treatments with nanoplant nutrient formulations due to high surface area of the nanoparticles of HA which leads to increased solubility of P. Although, previous studies have proven that plants are able to uptake nanoparticles through root system, the exact mechanism of phosphorous uptake is not well understood with respect to nanoparticles (DeRosa *et al.* 2010).

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