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Research Article

An Exploration into the Novel Synthesis of Gluconate Nano NPK Agricultural Fertilizers and its Consumptions in from Various Perspective

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Abstract

Aim: The present study aimed to synthesize the novel Gluconate nano NPK agricultural fertilizers and evaluate its efficacy in increasing soil's nutrient parameters.

Material and Method: Nano composites of nano Gluconate and chitosan with NPK source were prepared. Using X-Ray diffraction, and FTIR spectroscopy, the stability and interaction of Gluc-NP/CS NP suspensions containing fertilizers were assessed. An experiment involving laboratory incubation was used to measure the release of nutrients.

Result: The components were aggregated and loaded on the surface of biofertilizers, as shown by the mean diameter increase of the nano Gluconate nanoparticles/Chitosan nanoparticles in suspension with fertilizer addition. In the first four weeks, NPK composite loaded biofertilizer was found to have a significantly greater percentage of mineral nitrogen (MN) than the control. Less fluctuation was seen for exchangeable K during incubation, indicating a rapid release.

Conclusion: The findings showed coordinated nutrient release by nano composites with enhancing the nutrient parameters of soil and thus will be used on experimental field in further study.

Keywords: Agriculture; Chitosan Nanoparticles; Fertilizers; Gluconic Acid; Hydroxyapatite Nanoparticles

Abbreviations

N: Nitrogen; K: Potassium; P: Phosphorous; Hap: Hydroxyapatite; STPP: Sodium Tripolyphosphate; CS NCPs: Chitosan Nanocomposites; DI: Deionized; XRD: X-ray Diffraction; FTIR: Fourier-transform Infrared Spectroscopy; KBr-Potassium Bromide; NH_4OAc: Ammonium Acetate Glu-Gluconate

Introduction

The development of useful nanomaterials in recent years has paved the way for fresh developments in biotechnology and agriculture. To reduce fertilizer usage and environmental impact, slow-release fertilizer use has emerged as a new trend [1]. Chitosan nanoparticles is an intriguing substance for application

in controlled release systems because of its polymeric cationic, biodegradable, bio absorbable, and bactericidal properties [2-5]. However, there have been no efforts to investigate the possibility for controlled release of chitosan and hydroxyapatite nanoparticles in urea fertilizers. Because of the synergistic effects from interfacial particle-particle interactions, combinations of nano components may result in multifunctional characteristics [6]. Due to their hydrophilic and biodegradability properties, i.e., advantageous in several applications, natural polysaccharides have gained interest in the creation of nanoparticles [7,8]. Due to their unique features, chitosan nanoparticles were created [9-12]. It has been widely investigated how to use naturally occurring polymers, including chitosan, as carriers for therapeutic protein and gene delivery systems.

Chemical substances known as fertilizers are used to promote plant growth [13]. Fertilizers are often administered through foliar spreading or through the soil. Nitrogen, phosphorus, and potassium are the three major nutrients that artificial fertilizers give in the right amounts and combinations for a variety of crops. Nitrogen (N) promotes the development of proteins and chlorophyll in leaves. P (phosphorus) improves the growth of fruit, flowers, and roots. Protein synthesis and development of stems and roots are both induced by potassium (K) [14].

In underdeveloped nations, the price of fertilizers may be high and is often a constraint on the availability of food. It is crucial to create technologies that reduce the fertilizers costs by delivering them effectively and precisely. Because it contains a lot of nitrogen, urea is a frequent fertilizer. The conventional use of HA as a phosphorus fertilizer is limited by its poor solubility [15,16]. As a result, achieving P solubility through an NP formulation has an enhanced likelihood [17-19].

In the current study, the nitrogen, potassium, and phosphorus sources used to prepare the mineral composite included Gluconic acid, ammonia solution, hydroxyapatite, and potassium chlorate. The results could be extremely important in replacing less soluble chemical fertilizers as the nanocomposites provide better solubility and penetrability.

Materials and Methods

All of the following products were bought from Sigma-Aldrich: ethanol, acetic acid, hydroxyapatite (HAp), Gluconic acid, amino acid powder, potassium chlorate, and sodium citrate. From a Vietnamese firm, chitosan was purchased. Hi Media in Mumbai, India provided the sodium tripolyphosphate (STPP, 99%). For 1-2 hours, every chemical was subjected to sonication.

Preparation of chitosan nanoparticles

To make chitosan solution, 0.5 g of chitosan was dissolved in 150 mL of 2% acetic acid. A quick and easy procedure was used to create chitosan nanocomposites (CS NCPs) utilizing STPP as a reducing agent. In a typical synthesis, 50 mL of chitosan solution (and 5 mL of STPP were combined and agitated for 60 min at 37°C. The solution was then redistributed in DI water after being centrifuged (15000 rpm: 30 min). This was done to eliminate any surplus. For further procedures, chitosan nano particle was kept at 5°C.

Synthesis of nano gluconate nanocomposites/chitosan nanocomposites

Different amounts of nano Gluconate nanoparticles solution (6 mL, 10 mL, and 14 mL) were added to 50 mL of chitosan nanoparticles (CS NPs) solution, and the mixture was agitated for 60 minutes at 37°C. For further usage, the resulting solution was centrifuged and redistributed in DI water.

Preparation of NPK nanocomposites loaded with biofertilizer

Three ethanol-cleaned glass Petri dishes were filled with a balanced 6 g of hydroxyapatite, Gluconate, and potassium chloride fertilizer each. Next, we sprayed them with a CS NPs solution. To get CS NPs loaded fertilizer, these particles coated with CS NPs were dried for 20 minutes at 60 degrees Celsius.

Characterizations of nanocomposites

X-ray diffraction (XRD) was used to determine the structure of the produced nanocomposites. FTIR was performed in KBr pellet at 37°C across the range of 500 to 4000 cm^{-1} to examine the functioning of the sorbent.

Experimental analysis

Soil samples collection

The topsoil from crops nearby factory area was collected. The topsoil was made apparent by clearing away the surface debris, which included leaves, twigs, stumps, and other items. Using a soil

auger, soil samples were randomly taken from 0 to 20 cm deep at predetermined sites and bulked to create a composite sample. To eliminate large fragments of surface materials, the composite sample was crushed and run through a 4mm sieve after being air-dried in the lab. The composite sample was divided into two halves, one for incubation experiments and the other for chemical characterization.

Chemical characterization of the soil before the incubation experiment begins

After extraction with 1M NH_4OAc, accessible P was carried by the Mehlich 1 technique [21], and mineral N and total nitrogen by the "micro-Kjedhal method" [22]. Using a glass electrode and a 1: 2.5 soil to water (salt) ratio, the pH of the soil was measured.

Laboratory experiment using composite samples

The NPK addition to the soil for the incubation tests is shown in table 1 along with the fertilizer composite's composition. A1 is Gluconate, and nano-Hydroxyapatite/soluble fertilizer was used in the formulation of A2-A6. As the quantity of Gluconate and nano-HA decreases, in the composites the quantity of soluble fertilizer rises from A2 to A6, whilst A7 represents conventional fertilizer.

Samples	Amounts of fertilizer (ml) supplied to 1 kilogram of soil
A1	390
A2	360
A3	320
A4	285
A5	255
A6	230
A7	225

Table 1: The composition of the formulated fertilizer composite and the amounts of N, P and K, in the treatments.

The soil was amended with the prepared fertilizer mixture (1 mm) at a ratio of 60 mg nitrogen/ kg, completely blended, and placed into plastic bags. Three times each of the treatments (mentioned in table 1) was carried out, with soil i.e., untreated (control). Field capacity was diluted with distilled water (30% w/w), bags were sealed, and the mixture was cultured for 16 weeks at 20 °C in the dark. Since the start of incubation, the amounts of mineral N, P, and K were assessed every two weeks. Where the feel technique was employed to determine the need, soil was maintained wet at field capacity during the incubation time by adding up deionised water. By regularly allowing aeration via the opening of plastic bags, aerobic conditions were maintained. At the time of sampling, each sample was split into two halves. Available N was removed from one part and measured. Before doing an analysis for total Nitrogen, phosphorus, and potassium, the remaining fraction was air dried.

Results and Discussion

The crystallographic planes (211), (202), (130), (102), and (002), of the CS NPs crystals were represented by polycrystalline diffraction rings in the electron diffraction pattern (Figure 1(a)) of the CS NPs. The nano Gluconate NPs and CS NPs loaded with biofertilizers XRD pattern (Figure 1) coincided well with the normal Gluconate (Glu-) pattern, with feature diffraction peaks at 2 = 25.88°, 31.88°, 32.28°, 34.08°, 39.78°, and 49.58° assigned as facets of (002), (102), (130), and (202); and (213). As an alternative, two distinct signals for CS NPs have been seen at 2=26.08° and 10.58°. Except for peak height and breadth, the signals at Nano Glu-NPs/CS NPs were extremely comparable to those at CS NPs. According to prior expectations for certain Glu-/CS ratios [23-25], the removal of signals identifying CS NP in Nano Gluconate NPs/CS NPs was due to the low doping level of CS NP in the composite. The maximum strength of the diffraction peak at 2 = 23.04° was seen in the diffraction peaks of Nano Glu-NPs/CS NPs loaded with biofertilizer, as shown in Figure 2. (c). It showed that the surface of the Nano Glu-NPs and CS NPs composites had a bond with the amino groups of the biofertilizer. Thus, was shown that the biofertilizer was effectively loaded onto the Nano Glu-NPs/CS NPs.

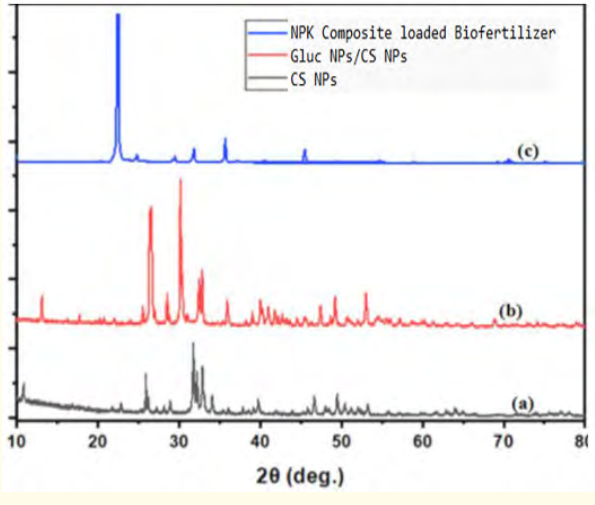

Figure 1: XRD patterns of (a) CS NPs; (b) Nano Glu-NPs/CS NPs; and (c) NPK nano composite loaded with biofertilizer, respectively.

As shown in figure 2 and table 2, most Nano Glu-NPs / CS NP signals generally corresponded to a significant degree with the primary Nano Gluconate signals. Intermolecular vibrations are those with frequencies between 2900 and 3444 cm^{-1}. However, the signals at "3420 cm^{-1} corresponding to the (-NH) stretching group only showed up in the biofertilizer-loaded CS NPs and Nano Glu-NPs/CS NPs. Additionally, the signals at 3318-3444 cm^{-1} and 1664-1728 cm^{-1} corresponding to (-OH) stretching and (-OH) bending groups only in Nano Glu-NPs/CS NPs and NPK nano composite loaded with biofertilizer but disappeared from CS NPs, which verify the NPK nano composite loaded with biofertilizer complex formation".

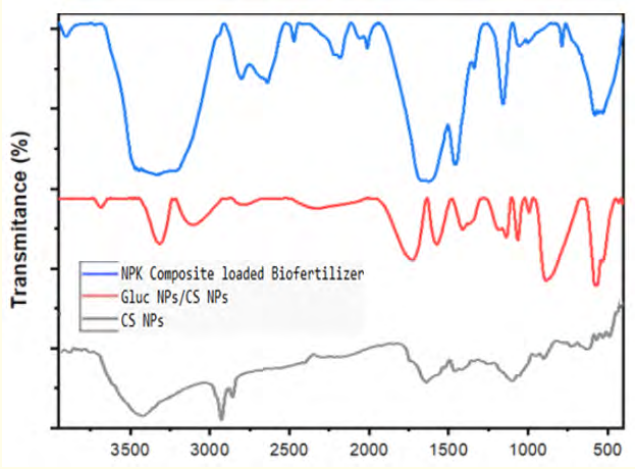

Figure 2: FTIR spectra's of (a) CS NPs, (b) Nano Glu-NPs/CS NPs and (c) NPK nano composite loaded with biofertilizer, respectively.

Characteristic group	CS NPs (cm^{-1})	Nano Glu-NPs/CS NPs (cm^{-1})	NPK nanocomposite loaded with biofertilizer (cm^{-1})
-NH (stretching)	3421	-	3420
-CH (stretching)	2900	2900	2900
-CH (bending)	1388	1388	1388
-CN	1643	-	1664
C - O - C (stretching)	1039	1039	1039
-OH (stretching)	-	3318	3444
-OH (bending)	-	1728	1664
-PO4	-	1066	1054
	-	888	788
	-	602	580
	-	566	558

Table 2: Characteristic FTIR wave numbers of CS NPs, Nano Glu-NPs/CS NPs and NPK nanocomposite loaded with biofertilizer.

The impact of varying amounts of chitosan nanoparticles solution on the dispersion in the H_2O with coated biofertilizer particles is clearly seen in figure 3. Details show that (A3) and (A4) samples exhibit significantly different attributes from (A1) sample and a blank sample. As a result, they cannot completely cover the core fertilizer. Diffusion's time order is thus just 43 minutes longer than the blank sample's time order of 4 minutes. In addition, the (A2) and (A6) sample can withstand 85 minutes in water, however the (A3) sample can only stabilize in 71 minutes. The cause is that when the fertilizer dries, rain dissolves it. As a result, the CS NPs solution of 10 mL was chosen as the ideal conditions for further studies.

The impact of the number of covering layers on the degradability of NPK composite loaded biofertilizer is shown in figure 4. The two-layer sample (A2) shows completely resistant water. The core

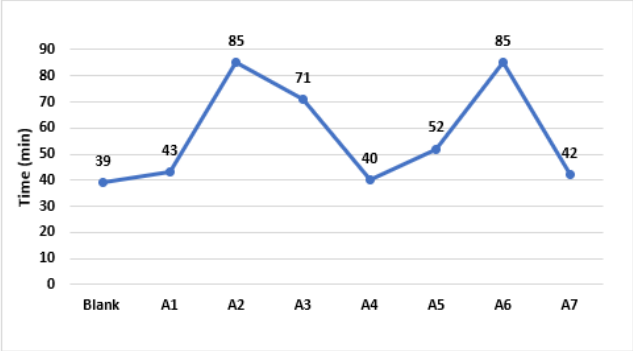

Figure 3: Effect of various volumes of NPK solution covered biofertilizer particles for diffusion in the water.

of the fertilizer is shielded for 118 minutes while submerged in deionised water. According to the samples (A1, A3, A4, A5, A6, A7 and blank samples), which were collected at times of 65 minutes, 84 minutes, 85 minutes, 76 minutes, and 36 minutes, either had three covering layers or none. Each fertilizer particle contains a significant amount of moisture when Nano Gluc-NPs/CS NPs is overloaded. As a result, when drying, a large amount of fertilizer was dissolved. However, the shells will readily absorb water if the NPK coating covering the bio fertilizer's surfaces is thin. Given that two layers are the most ideal situation to manage the NPK composite loaded biofertilizer surfaces.

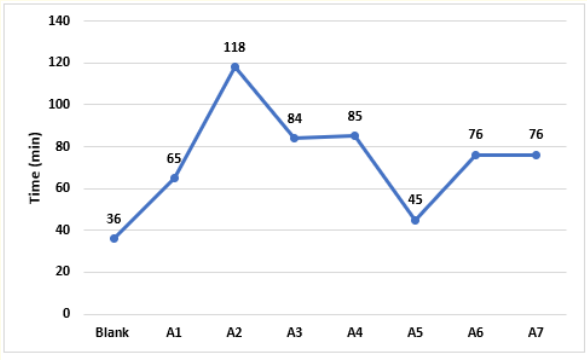

Figure 4: Effect various layer thicknesses of Nano Gluc-NPs/CS NPs solution covered on the biofertilizers' surfaces to dissolve time of the fertilizer in the water.

Chemical characteristics of soil at the onset of the experiment

Table 3 lists the most important properties of the study's soil. The site's soils had low available P content and were acidic. The central highlands' humid climate, which promotes the leaching of calcium, magnesium, and potassium as well as other basic cations, may be to blame for the soil's acidity. The low levels of accessible phosphorus might be linked to the acidity of the soil, which prevents phosphorus from being fixed and from being continuously removed by crops.

Parameter	Value
pH	5.25
Cation exchange capacity (C mol kg^{-1})	15.64
Available Phosphorus (ppm)	8.52
N (%)	0.29
Exchangeable potassium (C mol kg^{-1})	1.11
Mg (C mol kg^{-1})	4.26
Calcium (C mol kg^{-1})	8.51
Electrical conductivity (ds/m)	0.27

Table 3: Some important chemical characteristics of the soil utilized in the incubation experiment.

Nitrogen mineralization

Table 4 displays the mineral N concentration during the 16-week incubation period. The findings showed that MN content was low in the incubation early stages, decreased somewhat in the fourth week, increased significantly through the eighth week, peaked at the 12^{th} week, and at the 16^{th} week it was decreased subsequently. The lag phase, which is linked to the nutrients immobilization by microorganisms to feed as well as boost their biomass, is reflected in the Nitrogen's low mineralization in the early phases of incubation [26]. For maintenance and development, microorganisms need enough inorganic nutrients, water, trace elements, and carbon sources [27]. The fourth to the twelfth week corresponds to the microbial exponential growth phase, during which the bacteria have multiplied and are thus able to operate on the substrate. Even when they are fully satisfied, there are still more left over for the mineralization process, and this rate is considerably larger than that of immobilization.

Depletion of mineralizable substrate is the cause of the reduction in Mineral Nitrogen content after the 12th week. Additionally, the bacteria have exited the stationary phase and move into the endogenous growth phase, which would have decreased immobilization. Due to the sluggish nitrate absorption during establishment, rapid nitrate uptake throughout development and reproductive phases, and declining nitrate uptake during maturity, low Nitrogen mineralization found within the treatments up to 4 weeks may favour annual crops. Soils with clay minerals of type 2:1, including vermiculite and illite, are conducive to $NH4^+$ fixation because of the formation of the NH-O bond in the hexagonal pores and the balancing of a positive charge shortfall created by the isomorphous substitution of $Si4^+$ as well as $Al3^+$ ions [28]. An increase in focus and contact duration have both been demonstrated to improve fixation [29]. The release of amide-N may be responsible for the observed rise in MN concentrations in NPK fertilizer A1 to A6 between the eighth and sixteenth week.

Treatment	Incubation period (weeks)				
	2nd	4th	8th	12th	16th
A1	50.7	32.0	153.2	176.5	117.8
A2	58.0	45.1	177.4	201.1	127.4
A3	54.0	42.5	188.5	193.2	118.3
A4	55.3	46.0	205.1	266.1	139.1
A5	63.5	42.9	190.1	210.6	124.7
A6	58.3	48.8	211.7	242.1	131.2
A7	73.2	51.7	184.9	207.5	129.6
Control	43.7	23.9	107.4	145.9	85.5

Table 4: Mineral N(NO_3-N+NH_4-N) concentrations over the 16-week incubation period.

When added to organic fertilizer, MN from an inorganic source accelerates the degradation of organic matter [30]. Additionally, it has been observed that bacteria cultures originating from agricultural soils use PAM as a N source [31]. Pseudomonas putida H147, the bacterial strain the previous study, demonstrated 31.1% PAM degradation efficiency in 7 days and approached 45% under ideal conditions for growing. In contrast to acrylamide monomers, low molecular weight oligomer derivatives were visible in degraded PAM. Increased amounts of soluble Nitrogen from diammonium phosphate and readily hydrolysable Nitrogen from biofertilizer may be responsible for the perceived rise in the mineral nitrogen content in biofertilizer treatments A2 to A6. The microorganisms had easy access to a supply of nitrogen, but to get energy, the polymer needed to be broken down. Comparatively to the control, it has been shown that polymer medium supplied with mineral nitrogen, promotes PAM breakdown and microbial biomass [32].

The concentrations of ammonium-N and nitrate-N at various incubation stages are shown in table 5. In the second week, A7 had much more NH_4-N than A1, A2, A4, and A5, whereas in the fourth week of incubation, A7 had significantly more NO_3-N than A1, A3, A4, and A5. In the eighth, twelfth, and sixteenth weeks, there was no discernible difference between A7 and A2, A3, or A5, in terms of either NH_4-N or NO_3-N content; however, A4 and A6 had considerably higher values than A7. The much greater N concentration in A7 during the first two weeks of incubation reveals the existence of nitrogen, which the crops may not absorb entirely because it leaches out or gets stuck in clay. Better synchronization might, however, be achieved by preserving N in biofertilizer for later use.

Throughout the incubation, ammonium-nitrogen level was greater than nitrate-nitrogen content. The cumulative ammonium-nitrogen content then recorded higher values than nitrate-nitrogen content at the conclusion of the incubation period and typically rose from A1 to A6. There was no discernible difference between A7 and A2, A3, A4, and A5, or between A7 and A4, A5, and A6 in terms of cumulative NH_4-N concentration or cumulative NO_3-N content. The difference between the NH_4-N and nitrate-nitrogen contents explained by the soil acidity, which may prevent nitrifying bacteria from growing and carrying out their normal functions. Although there was no statistically significant difference between the treatments, there was a moderate rise in soil pH between weeks 12 and 16 of the incubation period, specifically in sites A2 to A6 (Table 6). The second week's pH reading of 5.15 was the lowest, while the sixteenth week's reading of 5.97 was the highest. It has been shown that soil pH ranges between 5.5 and 10.0, with an optimal pH value of approximately 8.5, are appropriate for the biological oxidation of NH_4^+ to NO_3 known as nitrification. The procedure is hampered at pH levels below 5 and performs best at pH levels above 6 [33]. Giroto., *et al.* (2017) in their study after 42 days of aerobic incubation "found that soil added with urea/HA and thermoplastic starch urea/HA additions had higher pH values than untreated soil, however the pH of soil amended with SSP, and HA stayed quite close to the control soil's pH (5). The excessive

urea hydrolysis in the soil with poor CEC and buffering capacity was blamed for the rise in pH in Nano-composite additions". It is advantageous to have more mineral nitrogen in the form of NH4-N since it is resistant to leaching losses [34].

Available phosphorous

Table 7 displays the available P at various incubation periods. The fourth week of incubation had the lowest Phosphorus values; the week eighth shows the highest, and the 12th and 16th weeks show virtually constant Phosphorus values. The immobilization of microbes and soluble Phosphorus adsorption into the soil might be the cause of the decrease in P concentration during the second and fourth weeks. P may have become more readily available after 4 weeks in all treatments due to the copolymer's breakdown and nano-microbial HA's solubilization. It has been shown that natural soil microorganisms may dissolve insoluble phosphates, including apatite.

In the soil and rhizosphere, "phosphate-solubilizing fungus (Penicillium, Aspergillus) and bacteria (Arthrobacter, Pseudomonas, and Enterobacter) are known to hydrolyze insoluble Phosphorus by secreting low molecular mass organic acids, to chelate mineral ions, or to reduce the pH" [35,36]. In addition to organic acids, mineral acids generated by chemoautotroph's and the H+ pump, such as those found in *Penicillium rugulosum*, have been observed to solubilize the Phosphorus [35]. As they travel further into the soil than bacteria do, soil fungi like mycorrhizae have been demonstrated to better solubilize Phosphorus. Additionally, the microbial cells' absorption of $NH4^+$ results in the release of H^+, which solubilizes P without the creation of organic acids. By substituting H^+ ions for Ca_2^+ ions, microbial cells acidification and the microbial cells surroundings induces the release of Phosphorus. The discharge of calcium ions into the soil may be what caused the pH of the soil to increase at the end of the incubation period (Table 5). Calcium ions have the effect of balancing the soil's acidity since they are bases [37].

Regardless of NPK formulas, fertilizer composites were measured to provide a specified quantity of nitrogen (50 mg N kg1) into the soil, hence the P content of the amendments varied as follows: "A2 = 68.1 mg kg1, A3 = 65 mg kg1, A4 = 64 mg kg1, A5 = 60 mg kg1, A6 = 58.5 mg kg1, and A7 = 54.5 mg kg1". From A2 to A6, the accessible phosphorus rose considerably ($p < 0.05$), which was explained by the presence of more soluble P and less hydroxyapatite. Except for the fourth week, there was no discernible difference between treatments A7 and A4, which may be ascribed to a balance between the quantity of P in each treatment and its availability in the soil. Since nitisols are powerful P scorpers [38], the fact that A7's P content was lower than that of A5's and A6's might be attributed to the soil's higher soil retention capacity, which rises with contact time [39,40]. The copolymer composite's encapsulation and slow microbial solubilization of the nano-HA may have decreased the amount of P time that was available for interaction in A5 and A6. Since P was absent from the shipments of the control and A1, there was no discernible difference between them. It was hard to determine the ideal quantity to be integrated into the fertilizer composite due to the amendments' variable P content.

Treatment	Incubation period (weeks)				
	2nd	4th	8th	12th	16th
A1	24.4	12.8	25.9	26.4	28.4
A2	24.4	12.6	39.2	37.9	41.2
A3	22.2	13.7	46.6	46.7	46.6
A4	26.0	14.3	55.1	54.2	51.7
A5	25.5	16.3	63.6	66.2	66.5
A6	27.5	16.4	76.2	80.3	76.7
A7	27.9	19.7	54.1	53.6	54.6
Control	21.0	10.3	25.2	23.8	26.5

Table 7: Content of available P (ppm) at different incubation times (weeks).

Exchangeable potassium

Table 8 displays exchangeable potassium at various incubation periods. During the incubation stage, the K concentration varied little, indicating a rapid release time. The high-water solubility of the K2SO4 salt and the tiny particle size of the SRF composite may have facilitated quicker K diffusion into the soil. In the first four weeks, there was no significant difference between A7 and SRF A1 to A6, but from the eighth to the sixteenth week, there was a significant

difference. Throughout the incubation period, the control did not deviate considerably from A1, which was anticipated given that K was not sent with the cargo. Similar to P, the quantities of K in the shipments varied (Table 1), making it difficult to determine the ideal quantity to be added to the fertilizer mixture.

Treatment	Period of Incubation (weeks)				
	2nd	4th	8th	12th	16th
A1	1.76	1.86	1.91	1.84	1.81
A2	1.88	1.84	1.99	2.04	2.07
A3	2.00	1.93	1.91	2.05	2.17
A4	2.06	2.04	1.94	2.00	1.94
A5	2.11	2.11	2.06	2.11	2.38
A6	2.16	2.12	2.26	2.18	2.28
A7	2.11	2.08	2.31	2.38	2.29
Control	1.64	1.71	1.86	1.59	1.71

Table 8: Concentrations of exchangeable K (C mol kg^{-1}) at different incubation times (weeks).

Conclusion

In the present study, nano Gluconate nanoparticles were effectively created at low temperature using a straightforward and environmentally benign approach. Additionally, the produced Gluc-nanoparticles successfully combined with chitosan nanoparticles (CS NPs). The synthesized NPK composite also loaded with biofertilizer can be used as a promising nanomaterials utilized in agriculture at the present time and in the future. The results, however, were based on lab incubation trials, and before they could be confidently suggested, an examination under actual field circumstances would be required.

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