

## Research Article

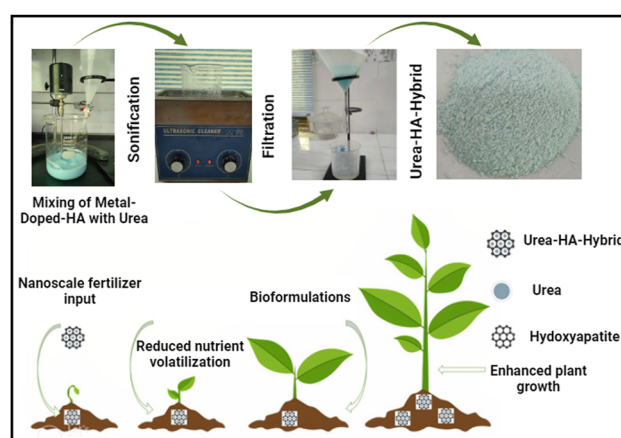
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# Advancing sustainable agriculture: Metal-doped urea–hydroxyapatite hybrid nanofertilizer for agro-industry

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**Abstract:** Nanotechnology holds excessive potential for addressing agricultural challenges such as soil deprivation, nutrient deficiencies, low harvests, and nutrient leaching. Nanofertilizers enable more efficient nutrient absorption by plants due to their enlarged surface area, bestowing viable solutions. Urea–hydroxyapatite hybrid (urea–HA hybrid) was successfully synthesized *via* a coprecipitation approach by doping nanohydroxyapatite with copper and zinc along with urea. The synthesized nanohybrids were analyzed by applying various techniques such as Fourier transform infrared spectroscopy, energy-dispersive spectroscopy (EDS), scanning electron microscopy, and X-ray powder diffraction (XRD). The evidence for the crystalline structure of HA was confirmed by peaks present in XRD analysis at 25.89°, 28.77°, and 32.11°, while urea was validated at 39.29°. The nanosized HA hexagonal nanorods were approximately  $16 \pm 1.5$  nm, with the incorporation of urea, Cu, and Zn. The components of urea–HA hybrid



Graphical abstract

(Ca, P, C, O, and N) were confirmed by EDS analysis with traces of Si. Antibacterial and antifungal activities were investigated against phytopathogenic microbes. The nanohybrid significantly inhibits the growth of *Clavibacter michiganensis*, *Xanthomonas campestris*, *Macrophomina phaseolina*, and *Sclerotium rolfsii*. A fertilization trial using urea–HA hybrid on *Citrus limon* has demonstrated a growth of 30 cm within 8 weeks of treatment, accompanied by brighter-colored leaves. Thus, the synthesized urea–HA hybrid enabled the slow release of nutrients, which had a significant impact on plant growth and will also effectively manage disease control against phytopathogens. Thus, this innovative approach addresses agricultural challenges regarding nutrient delivery and disease control more effectively.

**Keywords:** metal-doped-HA, micronutrients, nanofertilizers, urea–HA hybrid

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## 1 Introduction

The United Nations hopes to ensure food security by promoting more sustainable agriculture through the implementation of

the 2030 Agenda for Sustainable Development by highlighting the necessity of increasing food yields and effectively managing natural resources [1]. Fertilizers are considered to be significant components of soil nutrients that improve plant development and efficiency. Over the past 50 years, farmers have used many commercial or traditional fertilizers, which have balanced the circulation of the primary minerals required for the best growth of plants: potassium, nitrogen, and phosphorous [2–4]. Diammonium phosphate urea, rock phosphate, single superphosphate, nitrogen phosphorous potassium, and monoammonium phosphate are the most widely used conventional fertilizers and are rich sources of essential plant nutrients [5]. The global population is growing, as is the consumption of fertilizers. Despite the fact that plants can only absorb approximately 42% of the applied phosphorus, farmers are currently utilizing approximately 85% of the world's total mined phosphorus as fertilizer [6]. The use of these fertilizers causes significant economic losses in terms of 40–70% leaching-related concerns [7]. Additionally, heavy metals can cause serious harm to the ecosystem as well as to the soil microbial flora, soil structure, and plant life [8]. Therefore, the development of a special compost that gradually and economically discharges supplements is required so that the plants can effectively ingest the supplements [9]. The world's population will reach 9.6 billion by 2050, which will put more strain on the amount of arable land that can be used for farming. With current technological developments, food production and agricultural methods have reached a turning point; eventually, it would be almost impossible to feed the expected population. Sustainable agriculture urgently requires the development and implementation of smart agricultural methods using innovative technologies [10]. The scientific community has shown a great deal of interest in nanotechnology in the modern era because of its benefits in formulation design, analysis, and uses, which improve human safety and comfort [11–13]. However, given the widespread use of nanomaterials in many industries, it is essential to develop safer, more dependable, easy-to-use, non-toxic, and environmentally friendly methods for their manufacture [14,15]. Nanotechnology is a promising technique with tremendous potential for resolving agricultural-based issues such as land degradation, poor crop yield, nutrient shortage, and leaching losses [16,17]. The promising application of nanotechnology to enhance plant nourishment and reduce unfavorable ecological consequences is attracting much interest [18,19]. The soft release of essential molecules or ions from nanomaterials made of macronutrients is controlled by their rate and solubility. They differ significantly from conventional fertilizers as well as those of comparable bulk fertilizers. Additionally, because of their larger surface areas, they may be loaded with more macronutrients, such as nitrogen in the form of urea or nitrates, and have dissimilar profiles for kinetic release than their traditional counterparts.

Nanofertilizers have been found to have a high surface area, allowing plants to take in nutrients slowly and effectively, as desired [20,21].

Hydroxyapatite (HA), a mineral with a chemical composition of  $[(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2)]$ , is an essential component of both human and animal hard tissues, and because of its biocompatibility, HA is frequently employed as a bioceramic [22,23]. It contains calcium and phosphate ions, has high biocompatibility, and is commonly used in various biomedical applications [24–26], including drug and gene delivery, tissue engineering, bone mending, dental implants, and bioadsorption, but its use in agriculture is uncommon [27,28]. Naturally occurring rock phosphate, an allotrope of HA, is commonly employed as a phosphorus fertilizer despite its low solubility. However, it is possible to enhance its phosphorus solubility by modifying it into a nanoparticle formulation [29]. Therefore, one of the most promising alternatives to traditional fertilizers is the use of nanofertilizers [30]. In addition, the main goal of modern agricultural fertilization is to prevent nutrient losses and coordinate nutrient availability and plant uptake [31,32]. A blend of HA nanoparticles and urea, rather than urea alone, can be a rich nitrogen and phosphorus supplement due to its natural abundance, biodegradability, and high adsorption ability [33–35]. This selection was driven by multiple factors that collectively offer significant benefits for agricultural applications. These tailored materials are made of nanoparticles that contain macro- and micronutrients and distribute them in a controlled way to the rhizosphere of plants [36]. The urea-containing HA-based nanohybrids gradually release nitrogen into soil for a longer duration [37]. In addition, many micronutrients, including zinc, iron, boron, copper, and molybdenum, are necessary for the development of plants, and each has a specific use in agriculture [38–40]. Metal-doped HA stimulates the development of growth hormones, reproduction, photosynthesis, seed, grain formation, maturity, height, and protein synthesis [41]. However, zinc is a more crucial mineral since it helps to produce chloroplasts and growth hormones, while copper acts as an antimicrobial and antifungal agent for the production of chlorophyll [42]. Fertilizer prices can be enormous in developing countries, and they are usually a constraint on food production. Therefore, the development of effective and targeted fertilizer-delivery systems is essential to meet the ever-growing needs of mankind [43].

The core target of this study is to combine urea, copper, and zinc into HA nanocrystals to create a hybrid nanofertilizer that is sustainable and ecofriendly. Combining all these into a single hybrid material represents an innovative approach to its potential to provide a slow-release (nitrogen and phosphate), efficient multifunctional fertilizer that

enhances plant nourishment while addressing ecological concerns in agriculture. They reduce the frequency of fertilizer application by minimizing nutrient runoff. These nanohybrids were used to evaluate their possible antimicrobial action against bacterial and fungal phytopathogens for disease control management. These nanohybrids were employed in fertilization experiments to study the growth pattern of *Citrus limon*. The innovative aspect of this work is that the synthesized nanohybrids will not act as fertilizers but will also serve as disease-control agents.

## 2 Materials and methods

### 2.1 Chemicals

The chemicals with the highest purity were utilized in the study. Ammonium hydroxide ( $\text{NH}_4\text{OH}$ ), sodium bicarbonate ( $\text{NaHCO}_3$ ), orthophosphoric acid ( $\text{H}_3\text{PO}_4$ ), urea ( $\text{CO}(\text{NH}_2)_2$ ), calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ), copper chloride ( $\text{CuCl}_2$ ), and zinc chloride ( $\text{ZnCl}_2$ ) were procured from Sigma-Aldrich (China). All synthesis and measurements were carried out using deionized water throughout the experiment.

#### 2.1.1 Formation of metal-doped-HA

In the first step, using a coprecipitation technique, metal-doped-HA was synthesized by using  $\text{Ca}(\text{OH})_2$ , phosphoric acid,  $\text{NaHCO}_3$ , and metal salts. An aqueous solution of phosphoric acid (0.6 M, 250  $\text{cm}^3$ ) was incorporated slowly into the suspension containing  $\text{Ca}(\text{OH})_2$  (1 M, 250  $\text{cm}^3$ ), accompanied by 10 mL of 5%  $\text{ZnCl}_2$  and  $\text{CuCl}_2$ . The atomic ratio of

Ca/P in the precursor was set to be 1.67. The pH of the suspension was adjusted to greater than 10 using ammonium hydroxide.

#### 2.1.2 Formation of urea–hydroxyapatite hybrid (urea–HA hybrid)

In the second step, an aqueous solution of urea (2 M, 250  $\text{cm}^3$ ) was added to the suspension containing metal-doped-HA and sonicated for 2 h, as described in Figure 1. After which, the urea–HA hybrid suspension was filtered, repeatedly rinsed with deionized water, and dried at 70°C. Various batch-mode approaches were employed to optimize the synthesis and yield of this hybrid material at a laboratory scale. Experimental parameters were altered; pH and different ratios of urea, HA, zinc, and copper to find the optimal balance with the highest yield, without compromising the material's structural integrity and functional properties. The yield was quantitatively measured, and the purity of the hybrid nanocomposites was analyzed using X-ray diffraction (XRD) and scanning electron microscopy (SEM) to ensure that the optimization processes did not alter the desired properties of the hybrid.

## 2.2 Characterization techniques

### 2.2.1 Powder XRD

The XRD investigation of the nanohybrids was performed by using a Bruker AXS D4 Endeavour™ diffractometer. The continuous data scan for the urea-HA-hybrid was acquired

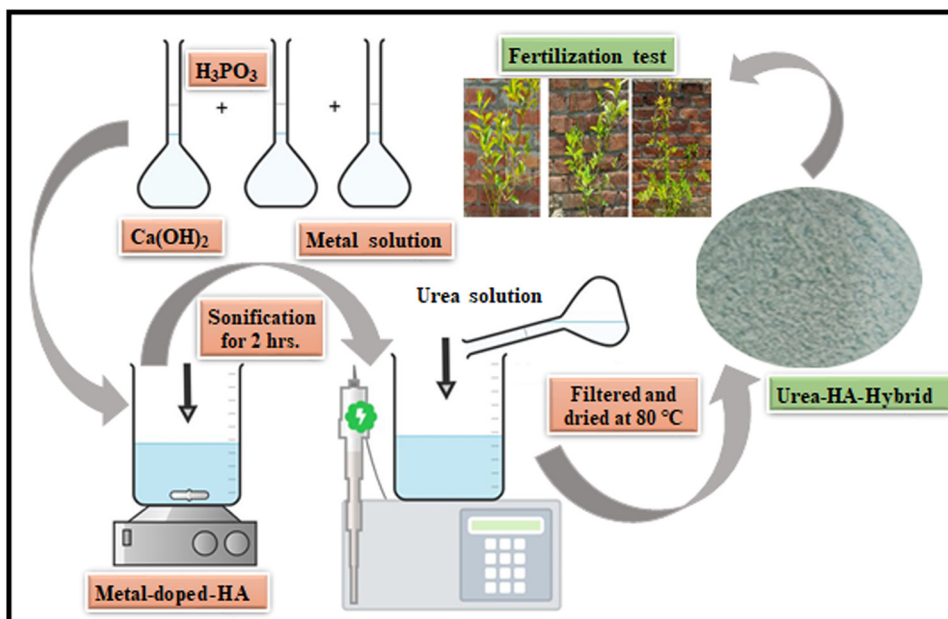


Figure 1: Scheme of Study for the synthesis of urea–HA hybrid.

over the  $2\theta$  range from  $20^\circ$  to  $80^\circ$  with a step size of  $0.05^\circ$  at 2 s/step by means of Cu-K $\alpha$  radiation where  $\lambda = 1.5406 \text{ \AA}$ .

### 2.2.2 Fourier transform infrared (FTIR) analysis

For the recognition of functional groups, FTIR spectra were obtained between  $650$  and  $4,000 \text{ cm}^{-1}$ , with a wave-number intermission of  $1 \text{ cm}^{-1}$ , 96 background, and sample scans by using ATR-FTIR (Agilent Technologies-Cary 360). The second derivative determination was carried out using Microlab Expert software provided by Agilent Technologies.

### 2.2.3 SEM-EDS

The elemental analysis, morphology, and particle size of the prepared metal-doped-HA and urea-HA hybrid was observed by SEM with EDS using a ZEISS GeminiSEM 360 instrument.

## 2.3 Antimicrobial activity

### 2.3.1 Antifungal properties

The two synthetic compounds, metal-doped-HA and urea-HA hybrid, were tested for their *in vitro* antifungal activity against *Macrophomina phaseolina* and *Sclerotium rolfsii*, two extremely harmful soil-borne fungal strains. The dry biomass reduction method was used for this purpose [38,39]. Each synthetic substance was weighed and dissolved in  $100 \mu\text{L}$  of dimethyl sulfoxide (DMSO) before the necessary amount of autoclaved malt extract (ME) broth was added to increase the content to  $6 \text{ mL}$ . This stock solution had a concentration of  $0.50 \text{ mg/mL}$ . A serial double dilution procedure was used to prepare lower concentrations ( $0.25$ ,  $0.125$ ,  $0.0625$ ,  $0.0312$ ,  $0.0156$ ,  $0.0078$ , and  $0.0039 \text{ mg/mL}$ ). The same quantity of DMSO that was present at various concentrations in the samples was used to generate a series of control treatments. Fungus inocula were prepared by adding sterilized distilled water to 8-day-old fungal colonies grown on ME agar plates. The experiment was carried out in three sets of  $5\text{-mL}$  test tubes using a random design. The growth media were provided in  $1 \text{ mL}$  per experimental and control tubes. As shown in Figure S1, each test tube received  $20 \mu\text{L}$  of fungal inoculum before being incubated at  $28^\circ\text{C}$  for 1 week. Before dry weight measurements, the fungal mates were filtered and dried at  $60^\circ\text{C}$ .

### 2.3.2 Antibacterial activity

*In vitro* antibacterial bioassays were undertaken by using different concentrations of metal-doped-HA and urea-HA hybrid against phytopathogens *Clavibacter michiganensis* and *Xanthomonas campestris* by using the paper disc diffusion method [44]. Luria Bertani (LB) agar was prepared by autoclaving at  $121^\circ\text{C}$  and  $103.4 \text{ kPa}$  for 30 min. Twenty milliliters of growth media were added to 90-mm diameter Petri test plate dishes and allowed to settle. A  $100 \mu\text{L}$  suspension ( $10^6 \text{ cfu mL}^{-1}$ ) of each bacterial species was spread on LB agar. Three concentrations of each synthesized compound ( $0.5$ ,  $1.0$ , and  $1.5 \text{ mg/mL}$ ) were prepared by mixing  $0.5$ ,  $1.0$ , and  $1.5 \text{ mg}$  of the compounds, respectively, in  $1 \text{ mL}$  of sterile distilled water. Sterilized filter paper discs ( $3 \text{ mm}$  diameter) were dipped in the solutions and placed on the surface of growth media inoculated with bacterial species. Distilled water was used as a negative control treatment, while the antibiotic ciprofloxacin ( $0.5 \text{ mg mL}^{-1}$ ) was used as a reference drug. The whole experiment was performed in a completely randomized design in triplicate. The plates were incubated for 24 h at  $37^\circ\text{C}$ , after which the zone of inhibition (mm) around the paper disks was measured.

### 2.3.3 Statistical analysis

Optimizing the concentration ensures that there is enough amount of nanocomposite suspension accessible for diffusion. To reduce the possibility of false negative results from diffusion variability, multiple experiments were carried out to validate the reproducibility of the findings. A comprehensive examination of the zone of inhibition data has been carried out, encompassing the rare instances when diffusion may have been lacking. There were also other quantitative techniques used, such as minimum inhibitory concentration testing and broth microdilution. By giving the nanoparticles and bacteria a more regulated environment to interact in, these techniques lessen the reliance on diffusion as the only mechanism of action observed. All the fungal biomass and bacterial growth inhibition data were analyzed by analysis of variance followed by the least significant difference (LSD) test ( $P \leq 0.05$ ) for separation of treatment means.

## 2.4 Fertilization test of urea-HA hybrid on plant growth

To investigate the potential of urea-HA hybrid, a fertilization experiment was conducted to evaluate the plant

growth. For this purpose, six *Citrus limon* were purchased from Punjab Horticulture Authority; two plants were given a dose of 50 plants mg/week of metal-doped-HA and urea–HA hybrid fertilizer; three plants were treated with 5 g/week of urea, dicalcium phosphate (DCP), and commercial fertilizer; the sixth plant remained untreated. All plants were treated for 2 months, and the length of each plant was measured before and after starting the dose each week with a measuring tape.

### 3 Results and discussion

The XRD pattern and crystalline nature of the urea–HA hybrid can be seen in Figure 2. The peaks at  $2\theta$  values of  $25.89^\circ$ ,  $28.77^\circ$ ,  $31.98^\circ$ ,  $32.86^\circ$ ,  $33.71^\circ$ ,  $46.74^\circ$ ,  $49.46^\circ$ , and  $53.33^\circ$  in the XRD pattern are associated with the crystalline HA nanoparticle reflection plane structure as standard JCPDS cards of HA (9-0432) [45,46]. The lattice parameters calculated for HA were  $a = b = 9.36 \text{ \AA}$  and  $c = 6.83 \text{ \AA}$ . The acquired data also demonstrated a noteworthy agreement with the standard ICDD database (01-074-0566) for HA [47,48]. The lower-intensity peaks visible at  $22.04^\circ$ ,  $28.96^\circ$ , and  $39.29^\circ$  correspond to urea, as confirmed by the JCPDS card no. 01-083-143 [17]. The incorporation of Cu and Zn into the HA structure did not reveal any additional peaks. This suggests that these metal ions were effectively integrated into the HA framework, resulting in the formation of a homogeneous HA phase [49]. According to the results, metal–ligand interactions between calcium in HA nanoparticles and nitrogen in urea represent major bonding sites [50].

FTIR spectroscopy was used for the identification of functional groups corresponding to the synthesized nanofertilizers, as exhibited in Figure 3. Structural modifications were observed in the nanohybrids as a result of replacing calcium ions with copper and zinc [51]. In metal-doped-HA and urea–HA hybrid, the P–O broadening of  $\text{PO}_4^{3-}$  ions was

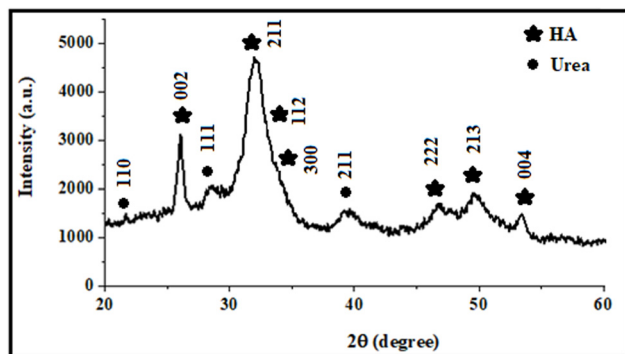


Figure 2: XRD of urea–HA hybrid.

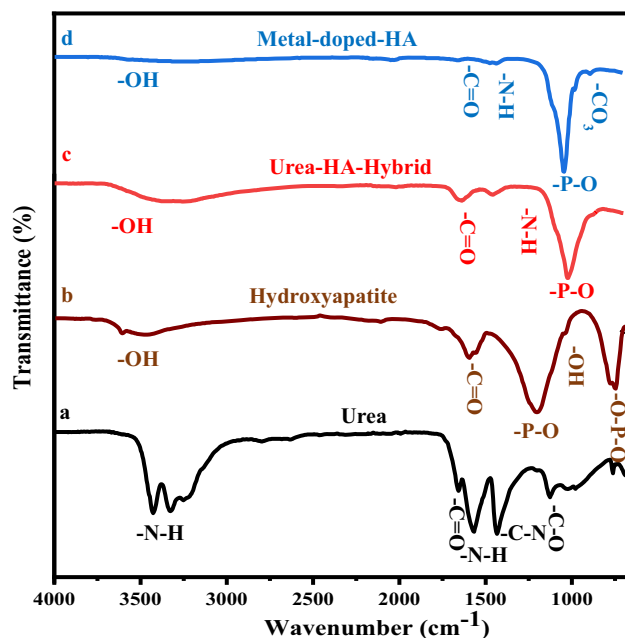


Figure 3: FTIR analysis of (a) urea, (b) HA, (c) urea–HA-hybrid, and (d) metal-doped-HA.

indicated by a strong peak at  $1,026$  and  $558 \text{ cm}^{-1}$  [52,53]. The NH bending motion of urea was observed at  $1,453 \text{ cm}^{-1}$  while urea exhibits primary amine characteristics, showcasing symmetric and asymmetric stretching of N–H bonds within the range of  $3,400$ – $3,250 \text{ cm}^{-1}$  as a double peak [54,55]. This suggests that the urea–HA hybrid mixture contains free NH bonds. In the urea–HA hybrid system, the C=O stretching vibrations are found at  $1,643 \text{ cm}^{-1}$  [56]. The bands at  $1,453$  and  $870 \text{ cm}^{-1}$  are associated with C–N [37,57]. The peaks at  $3,371$  and  $1,635 \text{ cm}^{-1}$  in metal-doped-HA and at  $3,329$  and  $1,643 \text{ cm}^{-1}$  in urea–HA-hybrid are attributed to the bending modes of absorbed  $\text{H}_2\text{O}$  [58]. The FTIR second derivative spectra of urea, HA, urea–HA-hybrid, and metal-doped-HA were obtained in the spectrum region of  $650$  to  $2,000 \text{ cm}^{-1}$  in order to improve accuracy, as shown in Figure 4. The peak located at  $961 \text{ cm}^{-1}$  is associated with  $\nu_1$  stretching mode of  $\text{PO}_4^{3-}$ . The distinctive bands detected at around  $1,022 \text{ cm}^{-1}$  are attributed to  $\text{PO}_4^{3-}$ 's  $\nu_3$  stretching vibrations. The vibrational modes  $\nu_2$  and  $\nu_3$  of  $\text{CO}_3^{2-}$  are associated with the bands located at  $873$  and  $1,415 \text{ cm}^{-1}$ , respectively, as exhibited in Figure 4(c) and (d). This is a characteristic band of HA of the B-type, where  $\text{CO}_3^{2-}$  substitutes  $\text{PO}_4^{3-}$  as confirmed from previously reported literature [53,59].

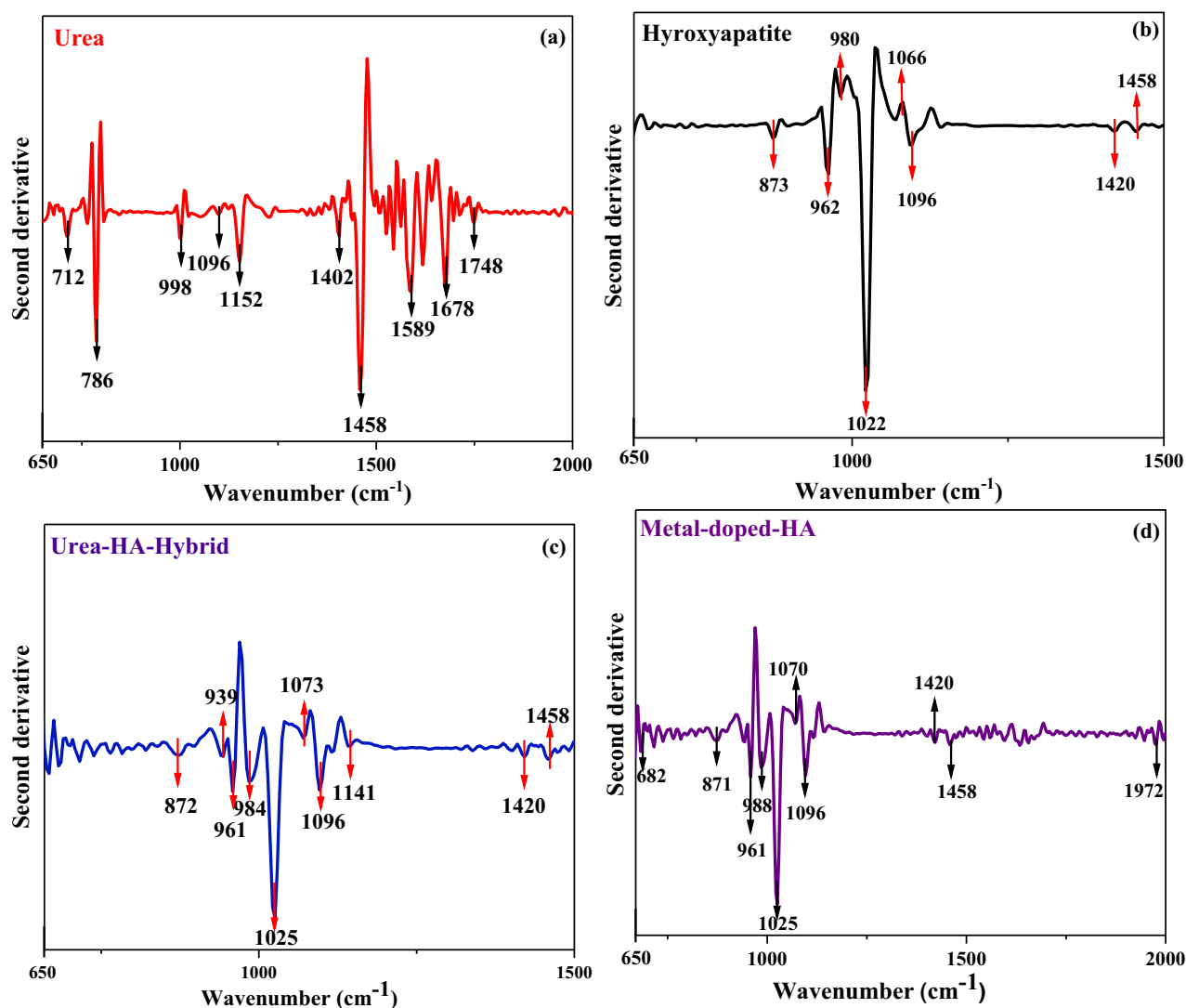
The morphology of the fabricated urea–HA-hybrid was examined by FE-SEM analysis. The study revealed that the nanourea particles had a fiber-like cloudy structure and were of various sizes, as confirmed by previous literature [2]. SEM micrographs have shown no discernible color or morphological variations, indicating consistent

and homogeneous mixing of urea and HA components, although they were clumped together owing to agglomeration. The particle diameter was estimated to be  $15 \pm 1$  nm, and the particles appeared as self-assembled hexagonal shapes of urea-doped HA nanorods. The inset of Figure 5a displays a further magnified image of the SEM for the prepared material and more precisely demonstrates the creation of cloud/nanoclusters of urea–HA-hybrid particles.

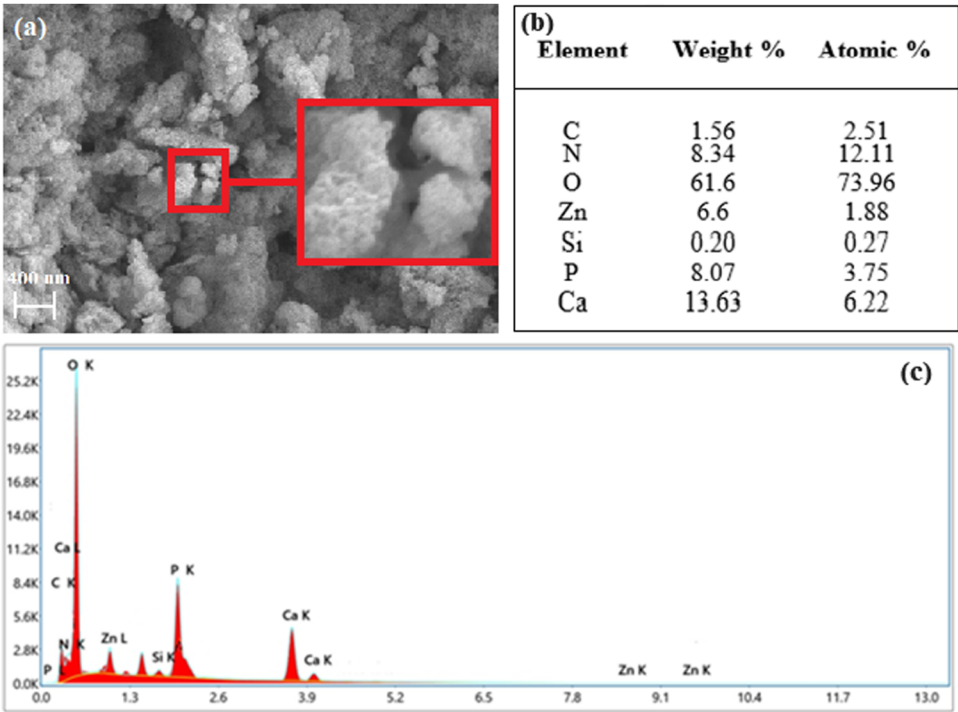
The elemental composition of urea–HA-hybrid was characterized and quantified by EDS analysis, as shown in sections b and c of Figure 5. This shows the presence of Ca, P, O, and N as major elements while Zn, C, and Si as minor elements, confirming the formation of urea–HA-hybrid. The Ca/P ratio was found to be 1.68, which is approximately similar to the theoretical value [60,61], while

the presence of nitrogen in urea–HA-hybrid is an indication that the amount of doped urea was 8.34% by weight. The nitrogen content was also counter-checked by using the Kjeldahl method and found to be in accordance with EDS analysis.

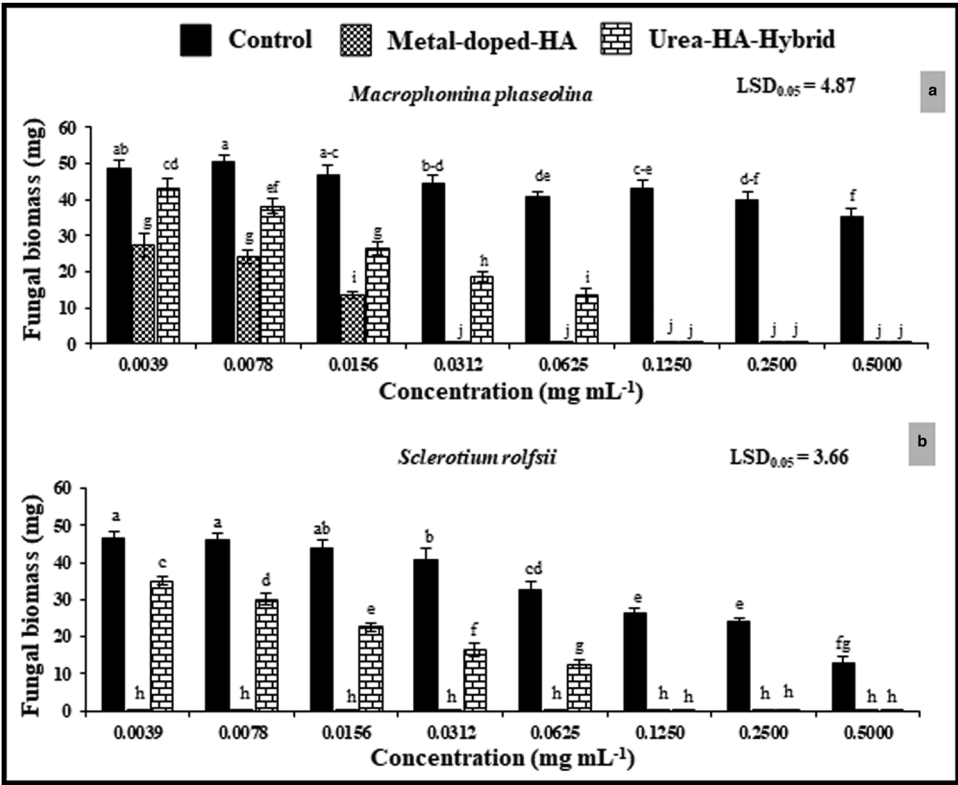
Plant health and growth can be negatively impacted by pathogenic microorganisms, which can result in illnesses and decreased yield. They may cause serious harm to plant tissues, causing necrosis, withering, and death, which would hinder the plant's ability to grow and develop as a whole [62]. Several strategical techniques, including crop rotation, the use of resistant plant cultivars, the use of biological control agents, and the application of fungicides or bactericides, are used to control these infections [63,64]. World-wide distribution of soil-borne fungi includes *M. phaseolina*,



**Figure 4:** Second derivative of (a) urea, (b) HA, (c) urea–HA-hybrid, and (d) metal-doped-HA.



**Figure 5:** Morphological and chemical characterization of urea-HA-hybrid: (a) SEM analysis, (b) elemental composition, and (c) elemental analysis by EDS.

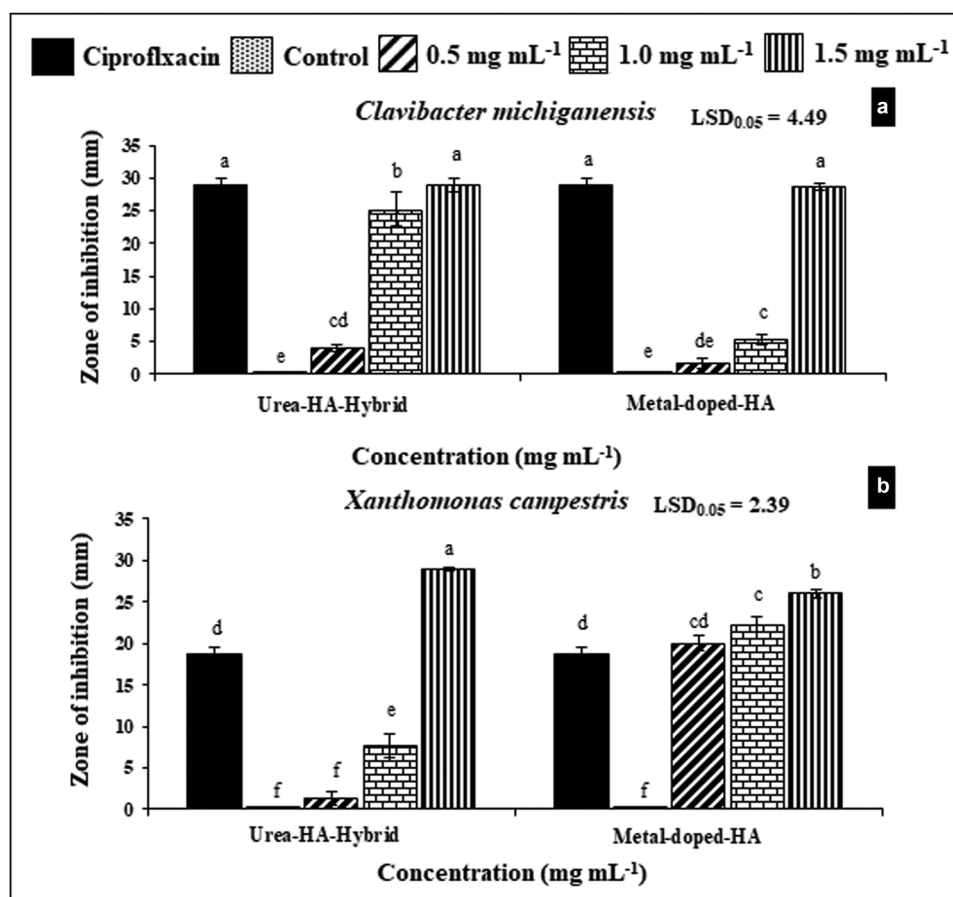


**Figure 6:** Antifungal activity of different concentrations of control, metal-doped-HA, and urea-HA-hybrid against (a) *M. phaseolina* and (b) *S. rolfsii*, the vertical bars represent the three replicate standard errors of means. According to the LSD test, values with different letters at the top demonstrate a significant difference ( $P \leq 0.05$ ).

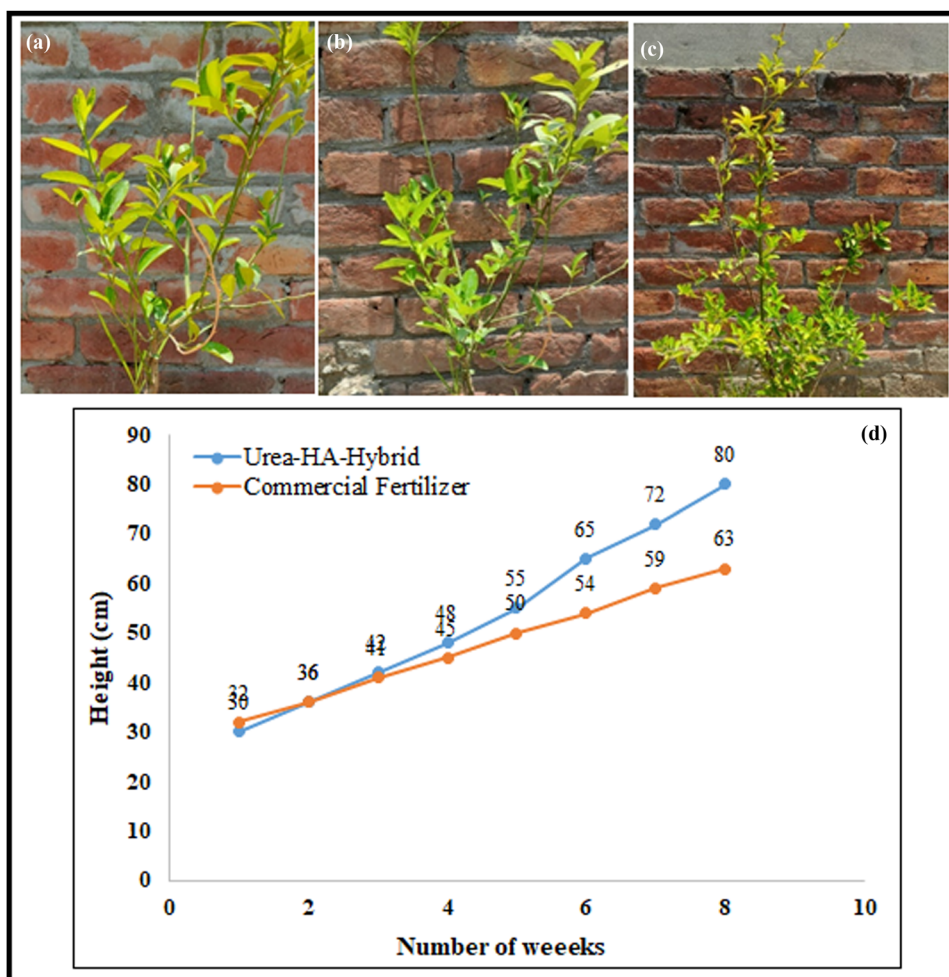
*S. rolfii*, *Aspergillus*, and *Fusarium* sp. They are the source of seedling blight, charcoal rot, and stem/root rot [65]. These fungi have the potential to significantly reduce agricultural yields. Figure 6 illustrates that both compounds exhibited potent antifungal activity against the target fungal species, indicating effective management of plant diseases. Additionally, the compounds demonstrated fertilizing abilities, enhancing their potential in agricultural applications. However, urea–HA hybrid was found to be far better than metal-doped-HA with respect to its antifungal activity. Lower concentrations (0.0039–0.015 mg/mL) of urea–HA hybrid reduced the biomass of *M. phaseolina* by 44–71%, while 0.0312 mg/mL entirely controlled the development of this fungal pathogen. Conversely, in the case of metal-doped-HA, concentrations ranging from 0.0039 to 0.0312 mg/mL lowered growth of *M. phaseolina* by 11–58%, as illustrated by Figure S2(a), while 0.0624 mg/mL completely controlled the fungal growth. *S. rolfii* was more susceptible to both the synthesized compounds than was *M. phaseolina*. All concentrations of urea–HA hybrid resulted in complete control of *S. rolfii*. In

contrast, fungal growth appeared in lower concentrations (0.0039–0.0625 mg mL<sup>−1</sup>) of metal-doped-HA and there was 25–61% growth suppression over the corresponding control treatments as shown in Figure S2(b). Only 0.1250 mg/mL and higher concentrations completely controlled the growth of this fungal species.

*C. michiganensis* is a Gram-positive bacterium that does not produce spores and thrives in aerobic conditions. It is known for its ability to harm tomatoes and potatoes, leading to significant economic damage globally [66]. Conversely, *X. campestris* is a Gram-negative plant pathogen that induces black rot disease in several cruciferous vegetables such as cabbage, cauliflower, and broccoli [67]. Both the synthesized compounds were effective against the two bacterial species. However, the effectiveness was highly variable with respect to type and concentration of the compounds, and the bacterial species *C. michiganensis* was more susceptible to urea–HA hybrid, while *X. campestris* growth was more sensitive to the application of metal-doped-HA. The reference antibiotic ciprofloxacin (10 mg/mL)



**Figure 7:** Antibacterial activity of antibiotic ciprofloxacin, control, urea–HA hybrid, and metal-doped-HA against (a) *C. michiganensis* and (b) *X. campestris*. The vertical bars represent the standard errors of the mean of three replicates. LSD test results show a significant difference ( $P \leq 0.05$ ) for values with various letters at the top.



**Figure 8:** Trial plant *C. limon* (a) untreated plant, (b) plant treated with commercial fertilizer, (c) plant treated with urea–HA hybrid, and (d) growth pattern of both the plants treated with urea–HA hybrid and commercial fertilizer.

caused 29 and 18 mm inhibition zones against *C. michiganensis* and *X. campestris*, respectively. Different concentrations of urea–HA hybrid (5, 10, and 15 mg mL<sup>−1</sup>) caused 4–29 and 1.3–29 mm inhibition zones against *C. michiganensis* and *X. campestris*, respectively. By contrast, metal-doped-HA application resulted in 1.7–28.6 and 20–26 mm inhibition zones against *C. michiganensis* and *X. campestris*, respectively (Figure 7). The zone of inhibition measurements demonstrated consistent diffusion from the disc, which was achieved by ensuring a standardized preparation procedure and precise incubation conditions.

The fungus becomes dormant and loses its ability to stick to fungal hyphae when the solution concentration increases due to high density. The nanohybrids inhibit fungal growth by rupturing cell walls and membranes, obstructing mycelial development and conidial germination, and generating reactive oxygen species (ROS) [68,69]. Urea–HA hybrid can interact with a variety of

biomolecules because of its strong ability to oxidize. Due to their potent and indiscriminate reactivity, these free radicals seldom diffuse far from the sites of formation. The bacterial cell structure is impacted by the presence of these nanohybrids, and when they come in contact with the bacterial cell, the rate of cell membrane destruction increases. According to certain hypothesized mechanisms, the nanoparticles permeate into bacterial cells and hinder their metabolic activities as a result of the production of ROS. They can also react with the cell wall and the membrane of bacteria or directly interact with bacterial DNA or thiol groups in cell proteins to halt all processes and result in cell death [44,70,71]. The phosphate ions from HA and nitrogen from urea released by the urea–HA hybrid are beneficial for plants, but they can cause osmotic stress in bacterial cells and an undesirable environment for certain pathogens. It is well known that zinc ions affect protein synthesis and bacterial enzyme activity. They may

interfere with the permeability of the bacterial cell membrane and prevent the synthesis of vital enzymes, which would restrict the growth of the bacteria [72,73]. ROS that cause oxidative damage to proteins, nucleic acids, and the bacterial cell membrane can be produced by copper ions [46,74].

The optimization processes have significantly enhanced the yield and quality of the urea–HA hybrid, ensuring that it is suitable for practical applications, particularly in the context of enhanced plant nourishment where efficacy and cost-effectiveness are matter of concern. The plants treated with urea–HA hybrid fertilizer were 30 cm in height in the first week, whereas those treated with commercial fertilizer were 32 cm in height. After 4 weeks of treatment, the plants treated with urea–HA hybrid showed substantial growth compared to the plants treated with commercial fertilizer, whereas the plant treated with commercial fertilizer shows modest increase in size and a minor change in color from green to yellow (Figure 8b). After 8 weeks, the plant treated with urea–HA hybrid outgrew the plant treated with standard fertilizer while no significant growth was observed in the untreated plant. The plant treated with nanohybrid grows not only in height but also in width, having a larger number of leaves with brighter color (Figure 8c). The comparison of the growth pattern of the plants treated with urea–HA hybrid, commercial fertilizer, and untreated is given in Figure 8(d). It is also evident from Table 1 that the urea–HA hybrid exhibits better performance in terms of enhanced plant growth and nutrient release efficiency. When urea, Zn, and Cu are added to the HA matrix, it becomes much more efficient and is therefore a better option for agricultural use than the other materials on the list.

The hybrid nanofertilizer improved the soil and water nutrient absorption ratios due to its smaller particle size as compared to the pore diameter of roots [2]. Plants fed with traditional fertilizer had a substantially lower concentration of these components. According to the calculated

results the test plant treated with urea–HA hybrid had a growth rate of 21.3%, while the one treated with traditional fertilizer had 15.7% as there was a difference of 17 cm in the height of both plants. The proportions of copper and zinc in hybrid fertilizer are 0.66 and 0.56, respectively, whereas these proportions are 0.26 and 0.06 by using traditional fertilizers as shown in Figure S3. According to the literature, functional nanohybrid materials exhibit multifunctional features caused by their synergistic properties from the interaction between particle interfaces [75]. The gradual discharge of urea from urea–HA hybrid causes a decline in the ratio of urea degradation in the soil, hence contributing to an enhanced nitrogen agronomic use efficiency [33]. In order to effectively promote nutrient uptake by plants, slow-release fertilizers employ a variety of techniques to release nutrients into the soil gradually. Over time, nutrients seep into the soil through the fertilizer matrix. Some nutrients are released by the mineralization of organic materials, while others are released from the coating by microbial activity in the soil or concentration gradients caused by changes in soil temperature and moisture. The mechanisms decreasing nutrient loss and increasing plant uptake efficiency, guarantee a more regulated and prolonged release of nutrients [76,77]. The release of nutrients from the urea–HA hybrid nanofertilizer occurs through diffusion and bacterial activity, particularly that of nitrifying bacteria.

## 4 Conclusion

Nanofertilizers can boost worldwide food production and support the growing global population. In the context of sustainable agriculture, they hold potential as a component of smart crop production systems because of their enormous surface areas, progressive, and regular distribution of nutrients. This study has been concluded with the successful synthesis of metal-doped-HA and urea–HA hybrid as confirmed by FTIR, XRD, SEM, and EDS analyses. The particle size was found to be  $15 \pm 1$  nm. In terms of disease control management, urea–HA hybrid completely restricted the growth of *C. michiganensis*, *X. campestris*, and *S. rolfsii*. Urea–HA hybrid treated plants showed a growth rate of 21.3%, while those treated with conventional fertilizers exhibited 15.7%. The combination of these elements offered a controlled release of essential nutrients which promoted efficient nutrient uptake, and as a result improved plant health, making this hybrid a potential solution for sustainable agricultural practices. Future research will concentrate on assessing the toxicity, bioavailability, and safety aspects of this nanofertilizers.

**Table 1:** Comparison of the urea–HA hybrid efficiency with other materials

| Material              | Nutrient release efficiency | Plant growth | Effectiveness |
|-----------------------|-----------------------------|--------------|---------------|
| Urea                  | Low                         | Low          | Low           |
| DCP                   | Low                         | Low          | Low           |
| Commercial fertilizer | Moderate                    | Moderate     | Moderate      |
| Metal-doped-HA        | Slightly high               | Moderate     | Moderate      |
| Urea–HA hybrid        | High                        | Very high    | Excellent     |

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## References

- [1] Carmona Fernández FJ, Ramírez Rodríguez GB, Delgado López JM. Urea-functionalized amorphous calcium phosphate nanofertilizers: Optimizing the synthetic strategy towards environmental sustainability and manufacturing costs. *Sci Rep.* 2021;11:3419. doi: 10.1038/s41598-021-83048-9.
- [2] Tarafder C, Daizy M, Alam MM, Ali MR, Islam MJ, Islam R, et al. Formulation of a hybrid nanofertilizer for slow and sustainable release of micronutrients. *ACS Omega.* 2020;5(37):23960–6. doi: 10.1021/acsomega.0c03233.
- [3] Dimkpa CO, Fugice J, Singh U, Lewis TD. Development of fertilizers for enhanced nitrogen use efficiency—Trends and perspectives. *Sci Total Environ.* 2020;731:139113. doi: 10.1016/j.scitotenv.2020.139113.
- [4] Bindraban PS, Dimkpa CO, Pandey R. Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. *Biol Fertil Soils.* 2020;56(3):299–317. doi: 10.1007/s00374-019-01430-2.
- [5] Lubkowski K. Environmental impact of fertilizer use and slow release of mineral nutrients as a response to this challenge. *Pol J Chem Technol.* 2016;18(1):72–9. doi: 10.1515/pjct-2016-0012.
- [6] Raliya R, Saharan V, Dimkpa C, Biswas P. Nanofertilizer for precision and sustainable agriculture: current state and future perspectives. *J Agric Food Chem.* 2017;66(26):6487–503. doi: 10.1021/acs.jafc.7b02178.
- [7] Ditta A, Arshad M. Applications and perspectives of using nanomaterials for sustainable plant nutrition. *Nanotechnol Rev.* 2016;5(2):209–29. doi: 10.1021/acs.jafc.7b02178.
- [8] Krasilnikov P, Taboada MA, Agriculture AJ. Fertilizer use, soil health and agricultural sustainability. *Agriculture.* 2022;12(4):462. doi: 10.3390/agriculture12040462.
- [9] Pahalvi HN, Rafiya L, Rashid S, Nisar B, Kamili AN. Chemical fertilizers and their impact on soil health. *Microbiota biofertilizers, Vol 2: Ecofriendly tools for reclamation of degraded soil environs.* Cham: Springer International Publishing; 2021. p. 1–20. doi: 10.1007/978-3-030-61010-4\_1.
- [10] Bollag J-M, Myers CJ, Minard RD. Biological and chemical interactions of pesticides with soil organic matter. *Sci Total Environ.* 1992;123:205–17. doi: 10.1016/0048-9697(92)90146-J.
- [11] Pansambal S, Oza R, Borgave S, Chauhan A, Bardapurkar P, Vyas S, et al. Bioengineered cerium oxide (CeO<sub>2</sub>) nanoparticles and their diverse applications: A review. *Appl Nanosci.* 2023;13(9):6067–92. doi: 10.1007/s13204-022-02574-8.
- [12] Cuong HN, Pansambal S, Ghotekar S, Oza R, Thanh Hai NT, Viet NM, et al. New frontiers in the plant extract mediated biosynthesis of copper oxide (CuO) nanoparticles and their potential applications: A review. *Environ Res.* 2022;203:111858. doi: 10.1016/j.envres.2021.111858.
- [13] Dabhane H, Ghotekar S, Tambade P, Pansambal S, Murthy HCA, Oza R, et al. A review on environmentally benevolent synthesis of CdS nanoparticle and their applications. *Environ Chem Ecotoxicol.* 2021;3:209–19. doi: 10.1016/j.jenceco.2021.06.002.
- [14] Kashid Y, Ghotekar S, Bilal M, Pansambal S, Oza R, Varma RS, et al. Bio-inspired sustainable synthesis of silver chloride nanoparticles and their prominent applications. *J Indian Chem Soc.* 2022;99(5):100335. doi: 10.1016/j.jics.2021.100335.
- [15] Narwal N, Katyal D, Kataria N, Rose PK, Warkar SG, Pugazhendhi A, et al. Emerging micropollutants in aquatic ecosystems and nanotechnology-based removal alternatives: A review. *Chemosphere.* 2023;341:139945. doi: 10.1016/j.chemosphere.2023.139945.
- [16] Segal D, Ciuffreda G, Mariotto G, Baldan B, Zamboni A, Varanini Z. FePO<sub>4</sub> nanoparticles produced by an industrially scalable continuous-flow method are an available form of P and Fe for cucumber and maize plants. *Sci Rep.* 2019;9(1):11252. doi: 10.1038/s41598-019-47492-y.
- [17] Marchiol L, Filippi A, Adamiano A, Degli Esposti L, Iafisco M, Mattiello A, et al. Influence of hydroxyapatite nanoparticles on germination and plant metabolism of tomato (*Solanum lycopersicum* L.): Preliminary evidence. *Agronomy.* 2019;9(4):161. doi: 10.3390/agronomy9040161.
- [18] Calabi-Floody M, Medina J, Rumpel C, Condron LM, Hernandez M, Dumont M, et al. Smart fertilizers as a strategy for sustainable agriculture. *Adv Agron.* 2018;147:119–57. doi: 10.1016/bs.agron.2017.10.003.
- [19] Servin A, Elmer W, Mukherjee A, De la Torre-Roche R, Hamdi H, White JC, et al. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. *J Nanopart Res.* 2015;17:1–21. doi: 10.1007/s11051-015-2907-7.
- [20] Butt BZ, Naseer I. *Nanofertilizers.* Nanoagronomy. Cham: Springer International Publishing; 2020. p. 125–52. doi: 10.1007/978-3-030-41275-3\_8.
- [21] Monreal CM, DeRosa M, Mallubhotla SC, Bindraban PS, Dimkpa C. Nanotechnologies for increasing the crop use efficiency of fertilizer-micronutrients. *Biol Fertil Soils.* 2016;52:423–37. doi: 10.1007/s00374-015-1073-5.
- [22] DileepKumar VG, Sridhar MS, Aramwit P, Krut'ko VK, Musskaya ON, Glazov IE, et al. A review on the synthesis and properties of hydroxyapatite for biomedical applications. *J Biomater Sci Polym Ed.* 2022;33(2):229–61. doi: 10.1080/09205063.2021.1980985.
- [23] Arokiasamy P, Al Bakri Abdullah MM, Abd Rahim SZ, Luhar S, Sandu AV, Jamil NH, et al. Synthesis methods of hydroxyapatite from natural sources: A review. *Ceram Int.* 2022;48(11):14959–79. doi: 10.1016/j.ceramint.2022.03.064.

- [24] Szcześ A, Hołysz L, Chibowski E. Synthesis of hydroxyapatite for biomedical applications. *Adv Colloid Interface Sci.* 2017;249:321–30. doi: 10.1016/j.cis.2017.04.007.
- [25] Dorozhkin SV. Multiphasic calcium orthophosphate (CaPO<sub>4</sub>) bio-ceramics and their biomedical applications. *Ceram Int.* 2016;42(6):6529–54. doi: 10.1016/j.ceramint.2016.01.062.
- [26] Gómez-Morales J, Iafisco M, Delgado-López JM, Sarda S, Drouet C. Progress on the preparation of nanocrystalline apatites and surface characterization: Overview of fundamental and applied aspects. *Prog Cryst Growth Charact Mater.* 2013;59(1):1–46. doi: 10.1016/j.pcrysgrow.2012.11.001.
- [27] Giroto A, Fidélis S, Ribeiro C. Controlled release from hydroxyapatite nanoparticles incorporated into biodegradable, soluble host matrixes. *RSC Adv.* 2015;5(126):104179–86. doi: 10.1039/C5RA17669G.
- [28] Jia X, Shi N, Tang W, Su Z, Chen H, Tang Y, et al. Nano-hydroxyapatite increased soil quality and boosted beneficial soil microbes. *Plant Nano Biol.* 2022;1:100002. doi: 10.1016/j.plana.2022.100002.
- [29] Kottegoda N, Sandaruwan C, Priyadarshana G, Siriwardhana A, Rathnayake UA, Berugoda Arachchige DM, et al. Urea-hydroxyapatite nanohybrids for slow release of nitrogen. *ACS Nano.* 2017;11(2):1214–21. doi: 10.1021/acsnano.6b07781.
- [30] Rop K, Karuku GN, Mbui D, Michira I, Njomo N. Formulation of slow release NPK fertilizer (cellulose-graft-poly (acrylamide)/nano-hydroxyapatite/soluble fertilizer) composite and evaluating its N mineralization potential. *Ann Agric Sci.* 2018;63(2):163–72. doi: 10.1016/j.aos.2018.11.001.
- [31] Gogos A, Knauer K, Bucheli TD. Nanomaterials in plant protection and fertilization: current state, foreseen applications, and research priorities. *J Agric Food Chem.* 2012;60(39):9781–92. doi: 10.1021/jf302154y.
- [32] Parra-Torrejón B, Cáceres A, Sánchez M, Sainz L, Guzmán M, Bermúdez-Perez FJ, et al. Multifunctional nanomaterials for bio-fortification and protection of tomato plants. *Environ Sci Technol.* 2023;57:14950–60. doi: 10.1021/acs.est.3c02559.
- [33] Gao X, Li C, Zhang M, Wang R, Chen B. Controlled release urea improved the nitrogen use efficiency, yield and quality of potato (*Solanum tuberosum* L.) on silt loamy soil. *Field Crop Res.* 2015;181:60–8. doi: 10.1016/j.fcr.2015.07.009.
- [34] Raguraj S, Wijayathunga WMS, Gunaratne GP, Amali RKA, Priyadarshana G, Sandaruwan C, et al. Urea-hydroxyapatite nanohybrid as an efficient nutrient source in *Camellia sinensis* (L.) Kuntze (tea). *J Plant Nutr.* 2020;43(15):2383–94. doi: 10.1080/01904167.2020.1771576.
- [35] Ammar M, Ashraf S, Baltrusaitis J. Nutrient-doped hydroxyapatite: Structure, synthesis and properties. *Ceramics.* 2023;6(3):1799–825. doi: 10.3390/ceramics6030110.
- [36] Nongbet A, Mishra AK, Mohanta YK, Mahanta S, Ray MK, Khan M, et al. Nanofertilizers: A smart and sustainable attribute to modern agriculture. *Plants.* 2022;11(19):2587. doi: 10.3390/plants11192587.
- [37] Sharma B, Afonso LO, Singh MP, Soni U, Cahill DM. Zinc-and magnesium-doped hydroxyapatite nanoparticles modified with urea as smart nitrogen fertilizers. *ACS Appl Nano Mater.* 2022;5(5):7288–99. doi: 10.1038/s41598-022-20772-w.
- [38] Palmer CM, Guerinot ML. Facing the challenges of Cu, Fe and Zn homeostasis in plants. *Nat Chem Biol.* 2009;5(5):333–40. doi: 10.1038/nchembio.166.
- [39] Du M, Chen J, Liu K, Xing H, Song C. Recent advances in biomedical engineering of nano-hydroxyapatite including dentistry, cancer treatment and bone repair. *Compos Part B: Eng.* 2021;215:108790. doi: 10.1016/j.compositesb.2021.108790.
- [40] Madusanka N, Sandaruwan C, Kottegoda N, Sirisena D, Munaweera I, De Alwis A, et al. Urea-hydroxyapatite-montmorillonite nanohybrid composites as slow release nitrogen compositions. *Appl Clay Sci.* 2017;150:303–8. doi: 10.1016/j.clay.2017.09.039.
- [41] Sadetskaya AV, Bobrysheva NP, Osmolowsky MG, Osmolovskaya OM, Voznesenskiy MA. Correlative experimental and theoretical characterization of transition metal doped hydroxyapatite nanoparticles fabricated by hydrothermal method. *Mater Charact.* 2021;173:110911. doi: 10.1016/j.matchar.2021.110911.
- [42] Abbasifar A, Shahrabadi F, ValizadehKaji B. Effects of green synthesized zinc and copper nano-fertilizers on the morphological and biochemical attributes of basil plant. *J Plant Nutr.* 2020;43(8):1104–18. doi: 10.1080/01904167.2020.1724305.
- [43] Sharma B, Afonso L, Singh MP, Soni U, Cahill DM. Zinc-and magnesium-doped hydroxyapatite-urea nanohybrids enhance wheat growth and nitrogen uptake. *Sci Rep.* 2022;12(1):19506. doi: 10.1038/s41598-022-20772-w.
- [44] Sadiqa A, Gilani SR, Anwar A, Mehboob A, Saleem A, Rubab S. Biogenic fabrication, characterization and drug loaded antimicrobial assay of silver nanoparticles using *Centratherum anthalmin-ticum* (L.) Kuntze. *J Pharm Sci.* 2021;110(5):1969–78. doi: 10.1016/j.xphs.2021.01.034.
- [45] Surmenev RA, Shkarina S, Syromotina DS, Melnik EV, Shkarin R, Selezneva II, et al. Characterization of biomimetic silicate-and strontium-containing hydroxyapatite microparticles embedded in biodegradable electrospun polycaprolactone scaffolds for bone regeneration. *Eur Polym J.* 2019;113:67–77. doi: 10.1016/j.eurpolymj.2019.01.042.
- [46] Predoi D, Iconaru SL, Predoi MV. Bioceramic layers with antifungal properties. *Coatings.* 2018;8:276. doi: 10.3390/coatings8080276.
- [47] Sahadat Hossain M, Tarannum S, Kawsar M, Bahadur NM, Ahmed S. Synthesis of pure and Cd-doped hydroxyapatite for the photo-catalytic degradation of Amoxicillin and Ciprofloxacin: Crystallographic characterization using XRD. *J Hazard Mater Adv.* 2024;13:100406–16. doi: 10.1016/j.hazadv.2024.100406.
- [48] Alam MK, Sahadat Hossain M, Bahadur NM, Ahmed S. Fabrication of composite scaffold using hydroxyapatite, epoxy resin and silica for load-bearing application. *Mater Adv.* 2024;5:6661–71. doi: 10.1039/D4MA00603H.
- [49] Sinulingga K, Sirait M, Siregar N, Abdullah H. Synthesis and characterizations of natural limestone-derived nano-hydroxyapatite (HAP): a comparison study of different metals doped HAPs on antibacterial activity. *RSC Adv.* 2021;11(26):15896–904. doi: 10.1039/D1RA00308A.
- [50] Kalita SJ, Bhardwaj A, Bhatt HA. Nanocrystalline calcium phosphate ceramics in biomedical engineering. *Mater Sci Eng: C.* 2007;27(3):441–9. doi: 10.1016/j.msec.2006.05.018.
- [51] Poinern GJ, Brundavanam R, Le XT, Djordjevic S, Prokic M, Fawcett D. Thermal and ultrasonic influence in the formation of nanometer scale hydroxyapatite bio-ceramic. *Int J Nanomed.* 2011;6:2083–95. doi: 10.2147/IJN.S24790.
- [52] Gritsch L, Maqbool M, Mourão V, Ciraldo FE, Cresswell M, Jackson PR, et al. Chitosan/hydroxyapatite composite bone tissue engineering scaffolds with dual and decoupled therapeutic ion delivery: Copper and strontium. *J Mater Chem B.* 2019;7(40):6109–24. doi: 10.1039/C9TB00897G.
- [53] Predoi SA, Ciobanu SC, Chifiriuc CM, Iconaru SL, Predoi D, Negrilă CC, et al. Sodium bicarbonate-hydroxyapatite used for removal of lead ions from aqueous solution. *Ceram Int.* 2024;50(1, Part B):1742–55. doi: 10.1016/j.ceramint.2023.10.273.

- [54] Zafar N, Niazi MBK, Sher F, Khalid U, Jahan Z, Shah GA, et al. Starch and polyvinyl alcohol encapsulated biodegradable nanocomposites for environment friendly slow release of urea fertilizer. *Chem Eng J Adv*. 2021;7:100123. doi: 10.1016/j.cej.2021.100123.
- [55] Predoi S-A, Ciobanu CS, Motelica-Heino M, Chifiriuc MC, Badea ML, Iconaru SL. Preparation of porous hydroxyapatite using cetyl trimethyl ammonium bromide as surfactant for the removal of lead ions from aquatic solutions. *Polymers*. 2021;13:1617. doi: 10.3390/polym13101617.
- [56] Ramirez-Gil JG, Lopera AA, Garcia C. Calcium phosphate nanoparticles improve growth parameters and mitigate stress associated with climatic variability in avocado fruit. *Heliyon*. 2023;9(8):18658. doi: 10.1016/j.heliyon.2023.e18658.
- [57] Xiong L, Wang P, Hunter MN, Kopittke PM. Bioavailability and movement of hydroxyapatite nanoparticles (HA-NPs) applied as a phosphorus fertiliser in soils. *Environ Sci: Nano*. 2018;5(12):2888–98. doi: 10.1039/C8EN00751A.
- [58] Xu J, Yang Y, Wan R, Shen Y, Zhang W. Hydrothermal preparation and characterization of ultralong strontium-substituted hydroxyapatite whiskers using acetamide as homogeneous precipitation reagent. *Sci World J*. 2014;2014:863137. doi: 10.1155/2014/863137.
- [59] Sroka-Bartnicka A, Borkowski L, Ginalska G, Ślósarczyk A, Kazarian SG. Structural transformation of synthetic hydroxyapatite under simulated in vivo conditions studied with ATR-FTIR spectroscopic imaging. *Spectrochim Acta Part A: Mol Biomol Spectrosc*. 2017;171:155–61. doi: 10.1016/j.saa.2016.07.051.
- [60] Vokhidova N, Ergashev K, Rashidova SS. Hydroxyapatite-chitosan *Bombyx mori*: synthesis and physicochemical properties. *J Inorg Organomet Polym Mater*. 2020;30:3357–68. doi: 10.1007/s10904-020-01649-9.
- [61] Pohshna C, Mailapalli DR. Engineered urea-doped hydroxyapatite nanomaterials as nitrogen and phosphorus fertilizers for rice. *ACS Agric Sci Technol*. 2021;2(1):100–12. doi: 10.1021/acsagritech.1c00191.
- [62] Jain A, Sarsaiya S, Wu Q, Lu Y, Shi J. A review of plant leaf fungal diseases and its environment speciation. *Bioengineered*. 2019;10(1):409–24. doi: 10.1080/21655979.2019.1649520.
- [63] Nazarov PA, Baleev DN, Ivanova MI, Sokolova LM, Karakozova MV. Infectious plant diseases: Etiology, current status, problems and prospects in plant protection. *Acta Nat*. 2020;12(3):46–59. doi: 10.32607/actanaturae.11026.
- [64] Pandit MA, Kumar J, Gulati S, Bhandari N, Mehta P, Katyal R, et al. Major biological control strategies for plant pathogens. *Pathogens*. 2022;11(2):273–94. doi: 10.3390/pathogens11020273.
- [65] Marquez N, Giachero ML, Declerck S, Ducasse DA. Macrophomina phaseolina: General characteristics of pathogenicity and methods of control. *Front Plant Sci*. 2021;12:634397.
- [66] Nandi M, Macdonald J, Liu P, Weselowski B, Yuan ZC. *Clavibacter michiganensis* ssp. *michiganensis*: Bacterial canker of tomato, molecular interactions and disease management. *Mol Plant Pathol*. 2018;19(8):2036–50. doi: 10.1111/mpp.12678.
- [67] Vicente JG, Holub EB. *Xanthomonas campestris* pv. *campestris* (cause of black rot of crucifers) in the genomic era is still a worldwide threat to brassica crops. *Mol Plant Pathol*. 2013;14(1):2–18. doi: 10.1111/j.1364-3703.2012.00833.x.
- [68] Huber A, Galgóczy L, Váradi G, Holzknecht J, Kakar A, Malanovic N, et al. Two small, cysteine-rich and cationic antifungal proteins from *Penicillium chrysogenum*: A comparative study of PAF and PAFB. *Biochim Biophys Acta - Biomembranes*. 2020;1862(8):183246. doi: 10.1016/j.bbamem.2020.183246.
- [69] Karpova NV, Yaderets VV, Glagoleva EV, Petrova KS, Ovchinnikov AI, Dzhavakhiya VV. Antifungal activity of the dry biomass of *Penicillium chrysogenum* f-24-28 and its application in combination with azoxystrobin for efficient crop protection. *Agriculture*. 2021;11(10):935. doi: 10.3390/agriculture11100935.
- [70] Rad Goudarzi M, Bagherzadeh M, Fazilati M, Riahi F, Salavati H, Shahrokh Esfahani S. Evaluation of antibacterial property of hydroxyapatite and zirconium oxide-modified magnetic nanoparticles against *Staphylococcus aureus* and *Escherichia coli*. *IET Nanobiotechnol*. 2019;13(4):449–55. doi: 10.1049/iet-nbt.2018.5029.
- [71] Verma KK, Song XP, Joshi A, Rajput VD, Singh M, Sharma A, et al. Nanofertilizer possibilities for healthy soil, water, and food in future: An overview. *Front Plant Sci*. 2022;13:865048. doi: 10.3389/fpls.2022.865048.
- [72] Kaur T, Putatunda C, Vyas A, Kumar G. Zinc oxide nanoparticles inhibit bacterial biofilm formation via altering cell membrane permeability. *Prep Biochem Biotechnol*. 2021;51(4):309–19. doi: 10.1080/10826068.2020.1815057.
- [73] El-Fallal AA, Elfayoumy RA, El-Zahed MM. Antibacterial activity of biosynthesized zinc oxide nanoparticles using *Kombucha* extract. *SN Appl Sci*. 2023;5(12):332. doi: 10.1007/s42452-023-05546-x.
- [74] Angelé-Martínez C, Nguyen KV, Ameer FS, Anker JN, Brumaghim JL. Reactive oxygen species generation by copper(II) oxide nanoparticles determined by DNA damage assays and EPR spectroscopy. *Nanotoxicology*. 2017;11(2):278–88. doi: 10.1080/17435390.2017.1293750.
- [75] Sanchez C, Boissiere C, Cassaignon S, Chaneac C, Durupthy O, Faustini M, et al. Molecular engineering of functional inorganic and hybrid materials. *Chem Mater*. 2014;26(1):221–38. doi: 10.1021/cm402528b.
- [76] Lawrencía D, Wong SK, Low D, Goh BH, Goh JK, Ruktanonchai UR, et al. Controlled release fertilizers: A review on coating materials and mechanism of release. *Plants*. 2021;10(2):238–63. doi: 10.3390/plants10020238.
- [77] Azeem B, KuShaari K, Man ZB, Basit A, Thanh TH. Review on materials & methods to produce controlled release coated urea fertilizer. *J Controlled Rel*. 2014;181:11–21. doi: 10.1016/j.jconrel.2014.02.020.