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Characterization of Calcium Phosphate Chitosan Nanocomposite as Plant Growth Promoter

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KEYWORDS

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Characterization

Plant growth stimulator

Plant growth promoter

ABSTRACT

In this study, calcium phosphate-chitosan nanocomposite (CaP-CS NC) was prepared by a convenient and affordable co-precipitation method, and the prepared NC was tested for agriculture application. Physico-chemicals analyses of the CaP-CS NC were conducted by X-ray diffraction (XRD), scanning electron microscope with energy dispersive X-ray spectroscopy (SEM-EDS), Fourier transform infrared spectroscopy (FTIR), and ultraviolet-visible spectroscopy (UV-Vis) instruments to determine the structural characteristics, surface topology, chemical composition, function group, and optical properties. The XRD pattern of CaP-CS NC revealed that the average crystallite size was 43 nm. The SEM images showed agglomeration of the CaP-CS NC with a rod-like shape. The EDS spectrum of the CaP-CS NC indicated the presence of Ca, P, O, and N elements. FTIR displayed vibrational peaks for the active functional group such as carboxylic (C=O), amines (N-H), hydroxyl (O-H), and alkyne (C-H). Furthermore, the spectrum of CaP-CS NC showed the bending mode of phosphates at 588.37 cm^{-1} and 508.45 cm^{-1}. The UV-Vis-NIR spectrum of the prepared nanocomposite indicates the anti-reflection properties, which might be useful in solar cell applications to increase the efficiency of the solar cell. In addition, the prepared CaP-CS NC was tested for the plant growth stimulator properties at the lab scale level, wherein it exhibited substantial growth. Accordingly, the current study suggests that the prepared CaP-CS NC could be used as a plant growth promoter.

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1 Introduction

Modern agriculture must adapt to ongoing climate change as well as rising food demand due to population growth (Shahrajabian et al. 2021). The exponentially expanding population, which is expected to reach 9.6 billion by 2050 with limited resources at present, compels the need for the development of sustainable agriculture. Increasing food production by 50% or more is urgently needed to meet the demands of an ever-growing population (Mittal et al. 2020; Yu et al. 2021; Husen 2021). The development of nanomaterials-based fertilizers paves way for positive effects such as regulated nutrient depletion rate, yield enhancement, crop protection, and minimizing post-harvest loss. On the other hand, nanocomposites are known as important materials that are made up of at least two components to maximize the benefits of each component. The shape and composition of the nanocomposites were improved with the chemical, physical, optical and biological characteristics of the primary matrix material in the first place (Ates et al. 2017; Salama 2017). This subsequently broaden the application areas of newly produced nanocomposites over single-applied nanomaterials (Esumi et al. 2003; Huang et al. 2004). Nanocomposite materials that emerged from polysaccharides have more advantages as they possess unique features such as biodegradability, biocompatibility, and bioactivity (Salama 2016; Salama et al. 2020).

Combining organic polymers like chitosan with inorganic minerals such as calcium phosphate is a viable strategy for creating novel composites with improved mechanical properties. Furthermore, porous scaffolds made from chitosan and inorganic minerals have been demonstrated and employed in a variety of sectors including agriculture (Salama et al. 2016). Chitosan solely has the potential to promote plant development in various crops and it boosts plant production while protecting them from diseases (Cita et al. 2018). It also has a considerable impact on root, shoot, flowering, and flower growth rates (Pandey et al. 2018). Calcium (Ca) and phosphate (P) are the key minerals for plants, since they aid in the metabolic process such as nutrient intake, promoting plant cell elongation, strengthening cell walls, and participating in enzymatic and hormonal processes (Upadhyaya et al. 2017).

However, the incorporation of chitosan oligosaccharide with key minerals like calcium phosphate to synthesize hybrid nanocomposite is with the expectance to exhibit growth-promoting ability. As chitosan and calcium both possess growth-promoting property individually. The application of nanocomposite in agriculture will bring sustainable agriculture systems (Kamle et al. 2020). As a result, it could open up new possibilities for producing new nanomaterials-based products (Husen and Siddiqi 2014). Based on its unique structural and biological properties, chitosan-calcium phosphate has gained a lot of attention among the explored sustainable biocomposite materials. Overall, this study aimed to highlight the efficient properties of prepared hybrid CaP-CS NC as a potential plant growth promoter to provide an effective solution to increase crop production sustainably.

2 Materials and Methods

2.1 Materials and synthesis of CaP-CS NC

Chitosan (CS), isopropyl alcohol, and acetic acid were purchased from Himedia, Mumbai, India. Calcium Phosphate nanoparticles(CaP NPs) were obtained from Nanotechnology Research Lab, Kongunadu Arts and Science College, India. The simple and cost-effective coprecipitation technique was used to formulate the calcium phosphate-chitosan nanocomposite (CaP-CS NC). About, 2 g of chitosan was dissolved in 50 mL of acetic acid, and the reaction vessel was stirred for 30 minutes, till it dissolves completely to get a perfectly transparent solution. Consequently, 4.5 g of previously developed CaP NPs were dissolved in 50 mL of isopropyl alcohol and stirred for 30 minutes. After that, the two solutions were mixed well and again stirred for 2 hours. The obtained white curdy solution of calcium phosphate nanoparticle was washed with deionized water two times and the prepared powder was dried at 100°C for 3 hours in a hot oven to remove water content. Later, it was calcined at 120°C for 5 hours to eliminate impurity in the prepared nanocomposite (Figure 1).

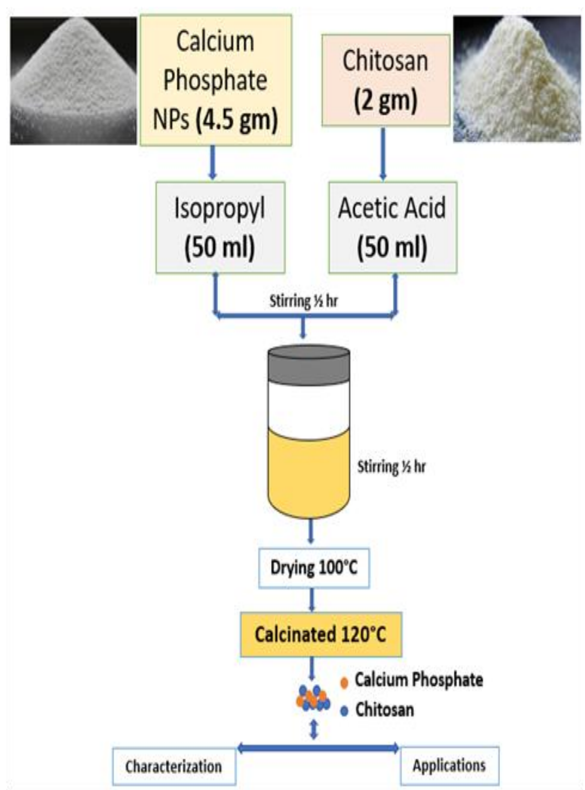


Figure 1 Schematic illustration of CaP-CS NC preparation

2.2 Physicochemical Characterizations of CaP-CS NC

To characterize CaP-CS NC, several physicochemical techniques including X-ray diffraction meter (XRD), Ultraviolet-visible spectroscopy (UV-Vis-NIR), Scanning electron microscope (SEM), and Energy dispersive X-ray spectroscopy (EDS) were used. The diffraction pattern of CaP-CS NC was analyzed using XRD (PAN analytics-BV) in the diffraction angle 2θ and range of 10°-80°. The surface topology image of CaP-CS NC was analyzed by SEM instrumentation (HITACHI SU-6000) and the elemental composition of CaP-CS NC was studied through EDX spectrum attached with SEM. The bonding linkage and functional group of prepared CaP-CS NC was studied by the FTIR spectrum. The optical characteristic of the prepared hybrid nanocomposite was analyzed through the UV-VIS spectrum (JASCO-V670, Japan).

2.3 Effect of CaP-CS NC on Black-eyed bean seed germination

About 100 mg of prepared CaP-CS NC was dissolved in 1000 mL of water. The prepared CaP-CS NC solution was used as a plant growth promoter. The black-eyed bean seeds (*Vigna unguiculata*) were purchased from the local market nearby the college campus. Collected seeds were washed with distilled water before being used. The normal plastic glasses (10 cm) were filled with about 10 g of soil (the soil sample collected from college gardening areas) and collected seeds were placed. The experiment was set with two different dosages (100 mL/day and 200 mL/day) of CaP-CS NC with control. The control experimental pot received only water and the other two experimental pots received CaP-CS NC solution of 100 mL and 200 mL per day. The whole experiment was set at the lab scale level. The CaP-CS NC treated and untreated seeds growth were measured and demonstrated. Figure 2 illustrates the schematic representation of nanocomposite treated and untreated seed germination.

3 Results and Discussion

3.1 XRD

The structure characteristic of CaP-CS NC was examined by XRD and measurements were carried out over the diffraction angle 2θ =10°- 90°(Figure 3).

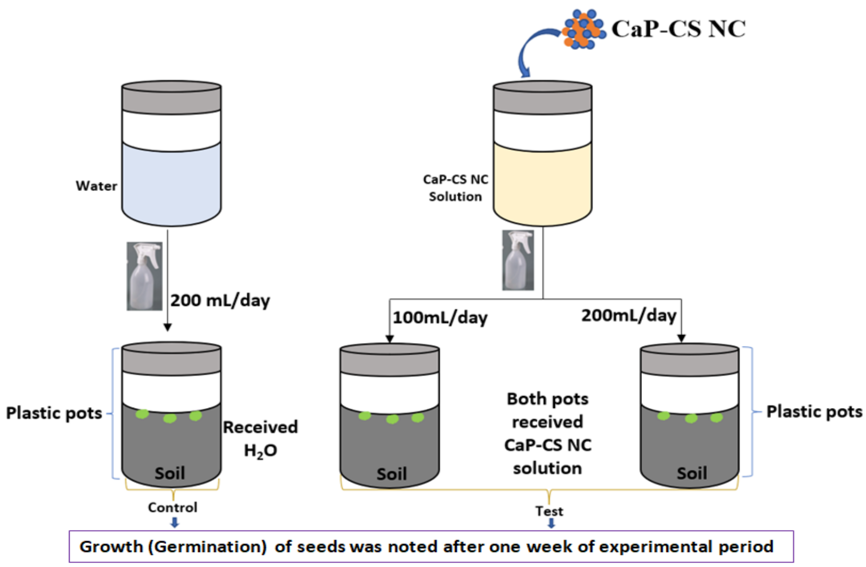


Figure 2 Schematic representation of CaP-CS NC treated and untreated seed germination

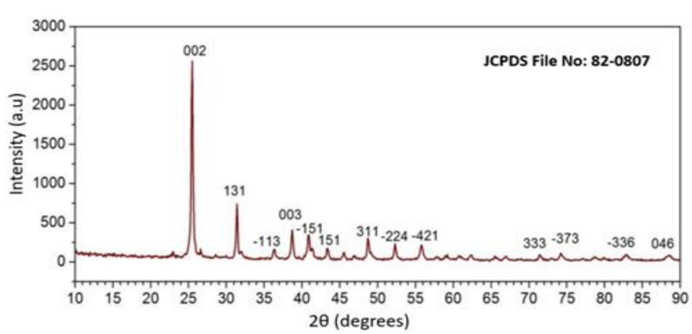


Figure 3 XRD spectrum of CaP-CS NC

Table 1 Structural parameters of CaP-CS NC

Hkl	2 θ (deg)	FWHM	Crystallite Size (D) (nm)	Dislocation Density Lines/meter
002	25.45	0.32	43.81	5.21
131	31.38	0.29	47.81	4.37
003	38.66	0.36	37.69	7.04

The diffraction peaks of CaP-CS NC were observed at 2θ values of 25.5°, 31.4°, and 38.70° corresponding to (002), (131), (003) orientation planes respectively, which indicates the hexagonal structure of the CaP-CS NC and it is in close agreement with the standard JCPDS file no 82-0807 which in turn implies that the prepared sample was free from impurities. The crystallite size of CaP-CS NC was estimated using Scherrer's formula (Manikantan et al. 2017). Table 1 shows the estimated structural characteristic of CaP-CS NC.

The average crystallite size of the CaP-CS NC was found to be 43 nm. In addition, the observed XRD characteristic peaks confirmed the crystalline nature of the prepared sample.

3.2 SEM-EDS

The surface topology of the CaP-CS NC sample was studied under SEM analysis (Figure 4). The SEM images showed the rod-like shape grains with a thickness of a few hundred nanometers of

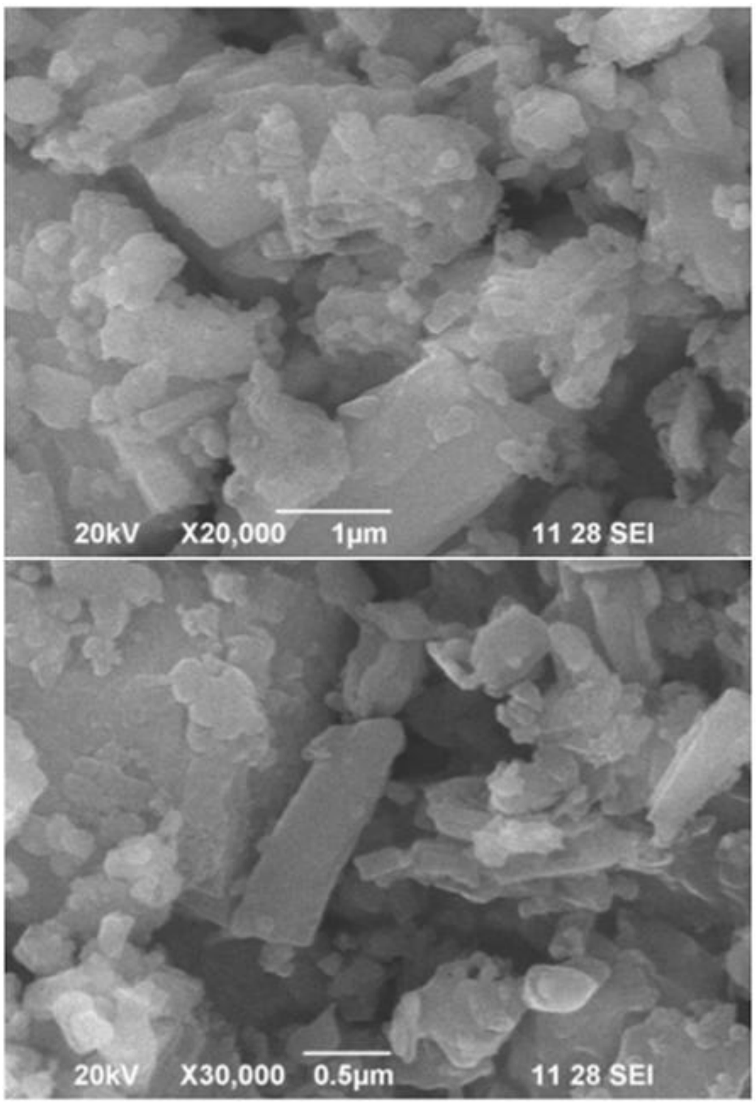


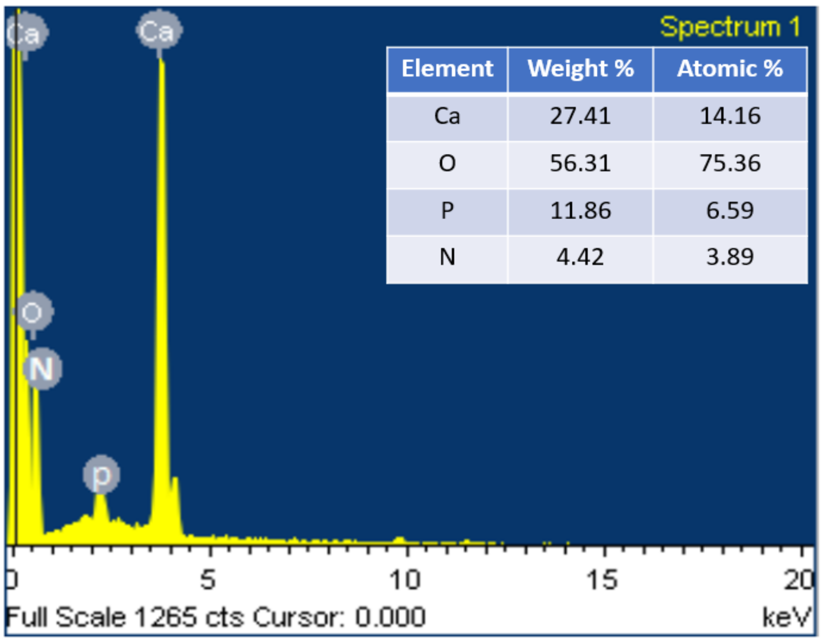
Figure 4 Surface topology of CaP-CS NC (different magnification level)

agglomeration of CaP-CS NC. The calcium phosphate nanoparticles reported by Milon and Tarakdas (2014) were about 45 nm in size with a spherical shape, and they have been utilized for biomedical applications.

The chemical composition of the prepared sample was carried out by EDAX to find out the presence of elements and their composition. It is obvious from the peaks that the product is composed of Ca, P, O, and N elements (Figure 5). The very weak P peak may be originated from the oxidation of the product exposed to the atmosphere air. The estimated elemental percentage of the prepared CaP-CS NC was 27.41 % (Ca), 11.86% (P), 56.31 % (O) and 4.42 % (N). Further, the EDAX spectrum revealed the absence of impurities in the synthesized nanocomposite (Peipei et al. 2010; Jun et al. 2013).

3.3 FTIR

FTIR spectrum of CaP-CS NC showed the characteristic peaks at various wavenumber regions revealing the presence and nature of vibration of elements in the nanocomposite. The FTIR spectrum showed the peaks at 3458.49 cm^{-1}, 2927.32 cm^{-1}, 2859.11 cm^{-1}, 2292.80 cm^{-1}, 1626.60cm^{-1},1153.30cm^{-1}, 676.55cm^{-1}, 622.13cm^{-1}, 588.37cm^{-1} and 508.45cm^{-1} respectively (Figure 6).



Element	Weight %	Atomic %
Ca	27.41	14.16
O	56.31	75.36
P	11.86	6.59
N	4.42	3.89

Figure 5 EDS spectrum of CaP-CS NC

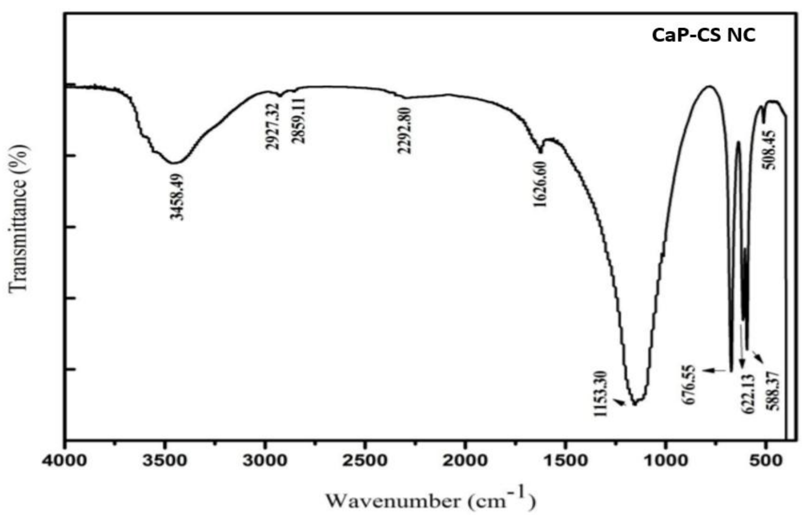


Figure 6 FTIR spectrum of CaP-CS NC

The peaks vibration at 3458.49 cm^{-1}, 1153.30 $cm^{-1}$and 622.13 cm^{-1} belong to the bending and stretching vibration of a hydroxyl group (O-H) of water molecules. This may be due to the hydroxyl atom present in the prepared sample or adsorption of atmosphere water molecules on the surface of a sample while recording the spectra analysis (Lesiak et al. 2019). The bands at 2927.32 cm^{-1}, 2859.11 cm^{-1}, and 2292.80 cm^{-1} are due to the occurrence of primary and secondary amines (N-H) and carboxylic group (C=O, O-H). The characteristic band at 1626.60cm^{-1} is a resultant of N-H stretching the function group of amines, which shows the presence of chitosan in the prepared nanocomposite (Cita et al. 2018). The peak at 622.13cm^{-1} is attributed to the bending of C-H due to alkyne group vibrations. The peaks at 588.37 cm^{-1} and 508.45cm^{-1} are due to bending modes of phosphates (Asep et al. 2019).

3.4 UV-Vis-NIR spectrum analysis

The reflectance spectrum of the prepared CaP-CS nanocomposite is presented in Figure 7.

The obtained spectrum showed that the reflectance first decreases with wavelength up to 400 nm and then it increases with an increase in wavelength. The average reflectance of about 60 % was obtained around 2000 nm. The observed high reflectance at higher wavelength regions indicates that these prepared CaP-CS NC might be applied as an anti-reflection layer in solar cells to enhance the efficiency of solar application (Huang et al. 2015). The optical properties of nanoparticles and nanocomposites such as transmission, absorption, light emission, and reflection are dynamic and may differ significantly from properties compared to the same kind of bulk material. The extensive range of optical effects of the nanomaterials may be produced for novel applications in the field of biomedical, electrical, food industry, cosmetic industry, solar, and agriculture sector by simply manipulating their size, shape, and surface functionality (Smith and Nie 2010; Daniel et al. 2012).

3.5 Effect of CaP-CS NC on Black-eyed bean seed germination

Nanotechnology can be used in agriculture crop production, animal feed, food processing, food contact materials, and food additives. In the agriculture sector, currently, researchers focus more attention on the development of novel nanofertilizers, nanopesticides, and growth biostimulators for improving productivity and suitability. In this study, an attempt has been made to utilize prepared CaP-CS NC as a seed germinator to develop a plant growth promoter to increase agricultural crop production. Figure 8 shows the seed germination of the black-eyed bean with the treatment of CaP-CS NC solution and water (control).

The present study revealed that at the end of day 7, the black-eyed bean seeds treated with CaP-CSNC solution of 100 mL/day and 200 mL/day exhibited a significant growth of shoot length of about 10 cm and 14 cm respectively. Whereas, the water-treated (control) sample showed 8 cm at the end of the experiment (7th day). Further, each of the pots were sown with three seeds of the black-eyed bean; all the three seeds germinated in the CaP-CS NC solution-treated pots, while only one of the seeds germinated in the water-treated pot. In addition, the root length and root branches were found to be increased with the increasing concentration of CaP-CS NC solution (Figure 8). Thus, the present study revealed that the prepared calcium phosphate-chitosan nanocomposite (CaP-CS NC) treated seed showed significant growth as compared to untreated seed.

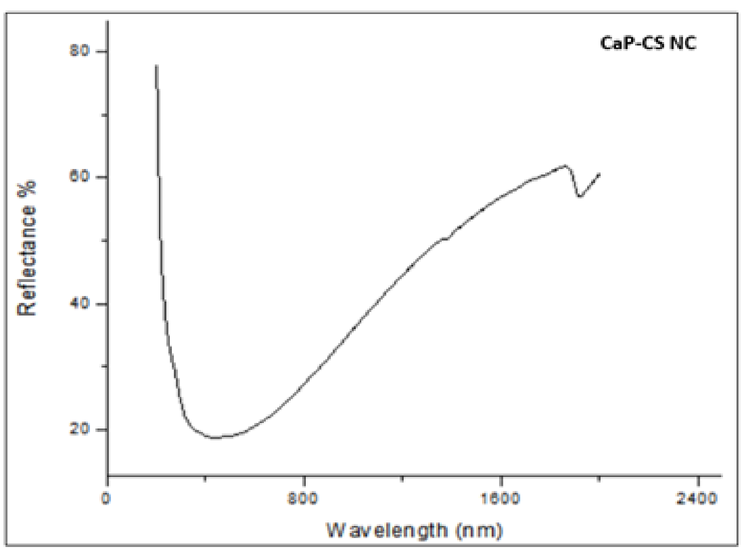


Figure 7 Reflectance spectrum of CaP-CS NC

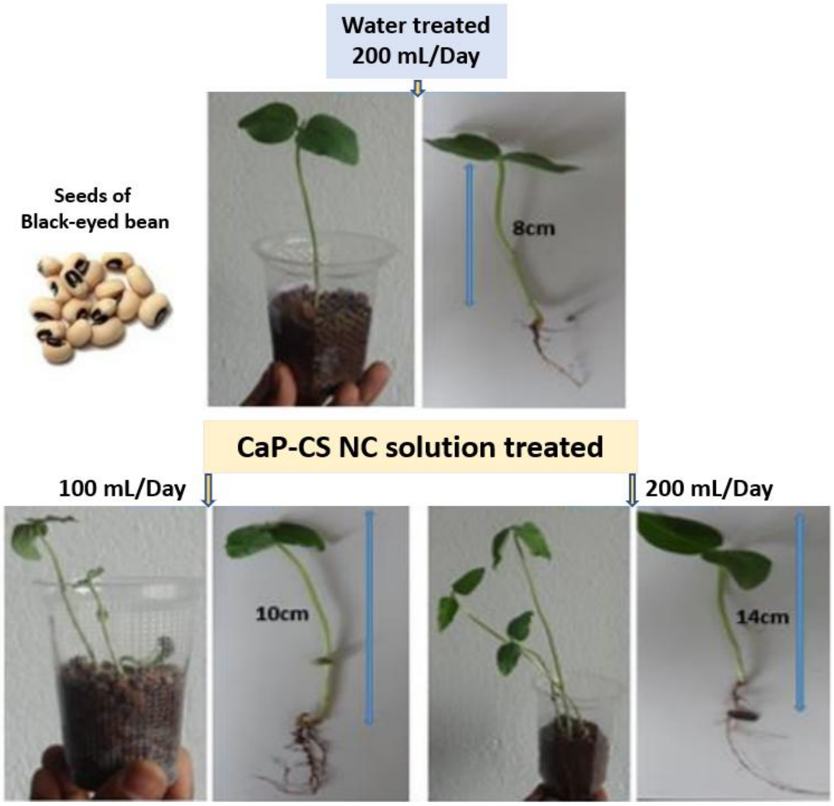


Figure 8 Effect of calcium phosphate - chitosan nanocomposite (CaP-CS NC) on Black-eyed bean seed germination (lab scale level)

Conclusion

The XRD spectrum confirmed the crystalline nature of the prepared CaP-CS NC and indicated size of 43 nm for the crystallite. The SEM images revealed agglomeration of the CaP-CS NC with a rod-like shape and EDAX instrumentation indicated the presence of elements such as Ca, P, O, and N in the prepared nanocomposite. The FTIR spectrum specified the different functional groups such as carboxyl, amine, and hydroxyl present in CaP-CS NC. UV-Vis-NIR exposed that the prepared nanocomposite might be used in solar cell applications as an anti-reflection layer to increase the efficiency of the solar cell. The prepared CaP-CS NC solution showed significant growth of black-eyed bean seeds at the lab scale level and it was observed that the growth directly depends on the concentration of the sample. Hence, the results of the present study revealed that the present nanocomposite could be used in the agriculture sector as a plant growth promoter. Further, it was revealed that calcium phosphate-chitosan nanocomposite could be used as plant growth promoters for high-yield agricultural crop production.

Conflicts of interest

The authors affirm that they do not have any conflict of interest

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