Int J Pharma Bio Sci 2017 October; 8(4): (B) 193-198

Original Research Article **Allied Sciences**

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

SYNTHESIS, CHARACTERIZATION AND PLANT GROWTH ASSESSMENT OF HYBRID CALCIUM OXIDE NANOPARTICLES

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ABSTRACT

Nanoscale materials exhibit novel chemical, physical and biological properties which are different from their bulk form. In the present study we have investigated synthesis and characterization of hybrid chitosan-calcium oxide nanoparticles for plant growth development in lab scale level. From the X-ray diffraction analysis the prepared nanoparticles shows polycrystalline nature with particles size varies between 1.97nm and 8.94 nm. Scanning electron microscopy revealed agglomeration of hybrid chitosan-calcium oxide nanoparticles. Energy dispersive X-ray spectroscopy analysis indicates that the prepared sample is composed of Ca, O and N elements. The plant growth progress in lab scale level revealed a significant growth of the Black eye beans plant growth after treatment of the hybrid chitosan-calcium oxide nanoparticle (8cm, 10.7cm) in comparison with untreated (6cm) after 7 days of experimental stage. The observation of the study revealed that hybrid chitosan-calcium oxide nanoparticles can be used to enhance the growth of Black eye bean plant and inferred that these nanoparticles may be used for other plants also.

KEYWORDS: ***Chitosan, calcium oxide nanoparticles, hybrid, Black eye bean plant growth.***

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Received on: 04-07-2017
Revised and Accepted on: 07-09-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.b193-198

INTRODUCTION

Chitosan is derived from thermochemical deacetylation of chitin found in the shells of crustacea and in some insects; chitosan is an abundantly available polymer. It is a natural polymer closely compared with cellulose. Chitosan is known to have a good complexing ability, the -NH_2 groups on the chain are involved in specific application[1]. Since, it has attracted attention because of its biological properties and effective uses in the medical field, food industries and agricultural sector[2]. Chitosan might be an effective antitranspirant to conserve water use in agriculture[3]. Ohta and coworkers (1999) reported that chitosan application to the soil at sowing time remarkably enhanced plant growth and flowered 15 days earlier than the control. Moreover, a greater number and weight of flowers were produced by chitosan treated plant[4]. Another study by Harada *et al.* (1995) indicated that the application of chitosan in the field increased shoot growth, branch length, and node number per plant and seed yield of soybean and total root length per plant increased by chitosan application in the pot experiment[5]. Different chitosan derivatives and enzymatic products have different structures and physicochemical properties, which may result in novel bioactivities or novel findings in known bioactive compounds. Due to its cationic character, chitosan presents a wide variety of physicochemical and biological properties, including antimicrobial, antioxidant and antihypertensive properties[6]. In general, chitosan offers several advantages and these include its ability to control the release of active agents and to avoid the use of hazardous organic solvents while preparing particles, nontoxic and biodegradable[7]. Nanoparticles have the potential to bring alteration in the agricultural development due to the unique physiochemical properties. Nanoparticles are also called as “magic bullets”, which contain nano-pesticide fertilizers, herbicides or genes, which aim to target particular cellular organelles in plant to pass their content. In addition NPs have a plant growth promoting effects, which have cosmic applications in agriculture[8-11]. A number of studies focus on developing physical and chemical strategies for synthesis of nanoparticles with controllable shape, size, morphology and magnetic properties. These are most important factors to consider in applications of nanoparticles[12]. On other hand, chitosan based nanoparticles have recently drawn much attention in nanotechnology due to their stability and alleviate of surface modification. The various techniques such as emulsion, self assembled, diffusion, ionic gelation, solvent evaporation, spray drying, cross linked and precipitation have been reported for the preparation of chitosan based nanoparticles[13]. Calcium is an essential plant nutrient that plays structural role in the cell wall and membranes and regulates plant growth and development[14]. Calcium oxide is an important inorganic compound, which is used across various industries as catalyst and toxic-waste remediation agent. Calcium oxide nanoparticles are of particular interest as it is regarded as a safe material to human beings and animals[15]. So far there is no report on the preparation and characterization of hybrid chitosan calcium oxide nanoparticles. So, in the present work an attempt has been made to study the synthesis, characterization and applications of hybrid chitosan calcium oxide nanoparticles for Black eyed bean plant growth development. To the best of our knowledge, this is first report on the growth of Black eyed bean plant using hybrid chitosan calcium oxide nanoparticles prepared by co-precipitation method.

Experimental techniques

Synthesis of hybrid chitosan-calcium oxide nanoparticles

The simple and cost effective co-precipitation method is used to prepare the hybrid chitosan calcium oxide nanoparticles. The chitosan was dissolved in a solution having 50ml of acetic acid and the solution was stirred at below 70ºC till it dissolves completely to get a perfectly transparent solution. Followed by 1M of an aqueous calcium chloride ($CaCl_2$) was added drop by drop to the transparent chitosan solution. Then 2M of an aqueous sodium hydroxide (NaOH) solution was added drop by drop till the transparent solution changes to white curdy solution. The solution was again stirred for 2 hours by maintaining the temperature (because chitosan is a biodegradable polymer). The obtained white curdy solution of chitosan capped calcium hydroxide nanoparticles was washed with deionized water and hydro-alcoholic suspension for ten times to remove sodium chloride (NaCl). This was done to enhance its stability which reduces the degree of agglomeration and improves the dispersion characteristics. The prepared powder was dried at 120ºC for 2 hours to remove the water contents and grinded by using mortar to get hybrid chitosan-calcium oxide nanoparticles.

Characterization of hybrid chitosan-calcium oxide nanoparticles

X-ray diffraction (Shimadzu, XRD 6000, Japan) was analysed for the range of 10° to 90° by powder X-ray diffraction using Cu-Kα radiation (1.5406 Å). FWHM (β) values and diffraction angles (θ) are used to analyse the crystallite size and peak broadening. The surface morphology of the prepared samples was characterized by Scanning Electron Microscopy (SEM) using GEOL JSM 6390 model. The stoichiometric composition of the prepared hybrid chitosan-CaO nanoparticles was analyzed by Energy Dispersive X-Ray spectroscopy (EDS) attached with SEM. The presences of the elements were identified by using EDS spectrum.

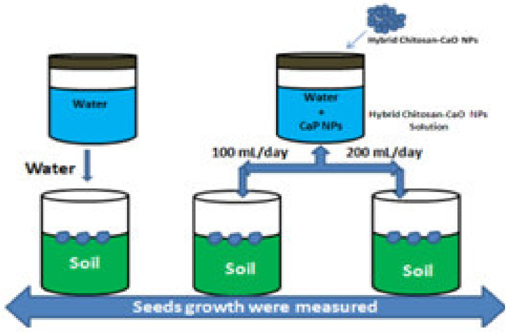
Hybrid chitosan-calcium oxide nanoparticles in plant growth

About 100mg of hybrid chitosan-CaO nanoparticles was dissolved in 1000mL of water. The prepared nanoparticles solution was used as plant growth promoter.

Treatment of nanoparticles solution in plant growth

The Black eye beans seeds were purchase from local market nearby Kongunadu arts and science college campus. Collected seeds were washed with distilled water before use. The normal plastic glasses were (10cm) filled with soil and collected seeds were sowed in soil. The experiment was set with two different concentrations of nanoparticles with control. The control glass receives only water and other two glasses were received nanoparticles solution 100 mL and 200 mL per day. The whole experiments were set in lab scale level.

The nanoparticles treated and untreated seeds growth was measured and presented in the photograph. Schematic diagram shows the experimental setup in lab scale level (Schematic-I).



Schematic I
Experimental setup for hybrid chitosan-calcium oxide nanoparticles on seed growth

RESULTS AND DISCUSSION

X-ray diffraction analysis

The XRD pattern of hybrid chitosan-calcium oxide nanoparticles is shown in Fig.1. The peaks observed at 2θ value of 26.77 and 25.49 respectively corresponds to (311) and (220) orientation planes. The absorbed peaks are in agreement with the standard JCPDS file no. 01-1160. There are many small peaks which indicate the polycrystalline nature of the hybrid chitosan calcium oxide nanoparticles. The crystallite size of prepared nanoparticles was calculated by using Scherrer's formula.

$$D = \frac{K\lambda}{\beta \cos \theta}$$

Where D = average crystallite size, K- shape factor (0.9), λ – wavelength of Cu Kα radiation (1.541 A°), β - Full Width Half Maximum (FWHM) of refection (in radians) located at 2θ and θ - angle of reflection (in degrees) was used to relate the crystallite size to the line broadening.

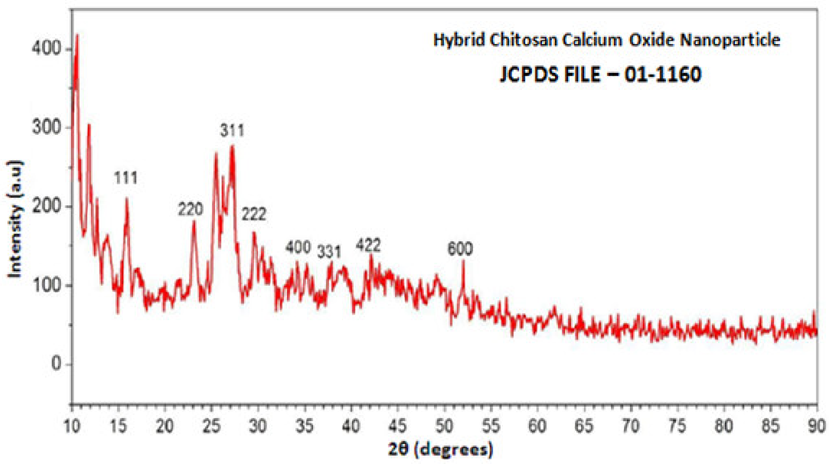


Figure 1
XRD spectrum of hybrid-chitosan calcium oxide nanoparticles

The crystallite size and dislocation density for the strongest three peaks were calculated and are presented in the Table I. The range of crystallite size varies from 1.97– 8.94nm. The small crystallite size obtained for hybrid chitosan-calcium oxide nanoparticles may be due to interaction of chitosan with calcium oxide.

Table I
Structural parameters of hybrid chitosan-calcium oxide nanoparticles

Hkl	2θ (degree)	FWHM (β)	Crystallite size(nm)	Dislocation density(lines/m) (10^{14})
311	26.7690	1.59530	8.94	12.524
220	0.72040	0.72040	1.97	2.568
111	10.5092	0.6009	2.33	1.862

Scanning electron microscopy analysis

The surface morphology of the hybrid chitosan-calcium oxide nanoparticles are shown in Fig.2.

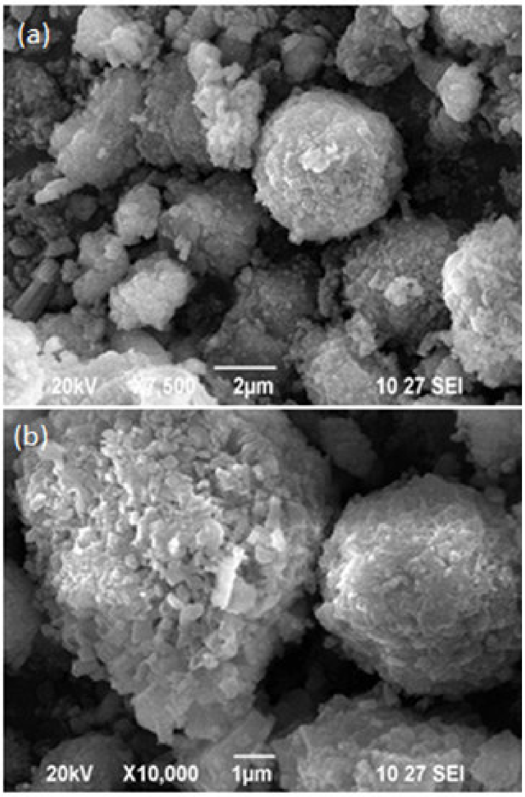


Figure 2
SEM images of hybrid-calcium oxide nanoparticles (a) magnified at 7,500 times and (b) magnified at 10,000 times

The bright area of the picture reveals high emission of secondary electrons when exposed to electron beam of SEM. This is due to high surface to volume ratio in those areas. The SEM images revealed agglomeration of hybrid chitosan-calcium oxide nanoparticles. It can be seen from the SEM image that the synthesized sample composed of grains of spherical shape. The agglomeration may be resulted from the interaction between nanoparticles with large surface area and high surface energy[16]. The image of the prepared sample magnified at 7,500 times indicated that the prepared sample consists of mostly sphere shaped grains (Fig.2a). The high magnification image (10,000 times) indicated that many micro pores exist in the exterior and interior of hybrid chitosan-calcium oxide nanoparticles (Fig.2b).

Energy dispersive X-ray spectroscopy analysis

The elemental compositions of the prepared nanoparticles were carried out by EDS for the evaluation of composition and purity.

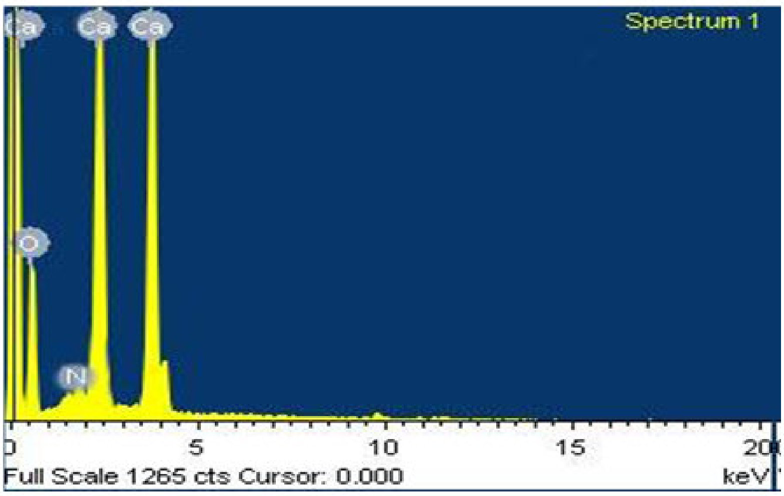


Figure 3
EDS analysis of hybrid-calcium oxide nanoparticles

The EDS spectrum of the hybrid chitosan-calcium oxide nanoparticles showed the prepared sample is composed of Ca, O and N elements (Fig.3). It is clear from the spectrum that the major peaks appeared for Ca and O. The ratio of chitosan-calcium oxide elemental composition (Table II) revealed the absence of any impurities in the prepared hybrid chitosan-calcium oxide nanoparticles.

Table II

Elemental composition of hybrid chitosan-calcium oxide nanoparticles

Element	Weight%	Atomic%
O	53.77	72.42
N (Chitosan)	3.27	1.72
Ca	42.96	25.86

Hybrid chitosan calcium oxide nanoparticles in plant growth development (Lab scale)

In agricultural field, for example, nanofertilizers, nanopesticides and nanosensors are being directly applied to agriculture soils in order to enhance crop productivity[17-19]. In the present study an attempt has been made to study the application of the prepared hybrid chitosan-calcium oxide nanoparticles as Black eye bean plant growth promoter (Fig.4a-c).

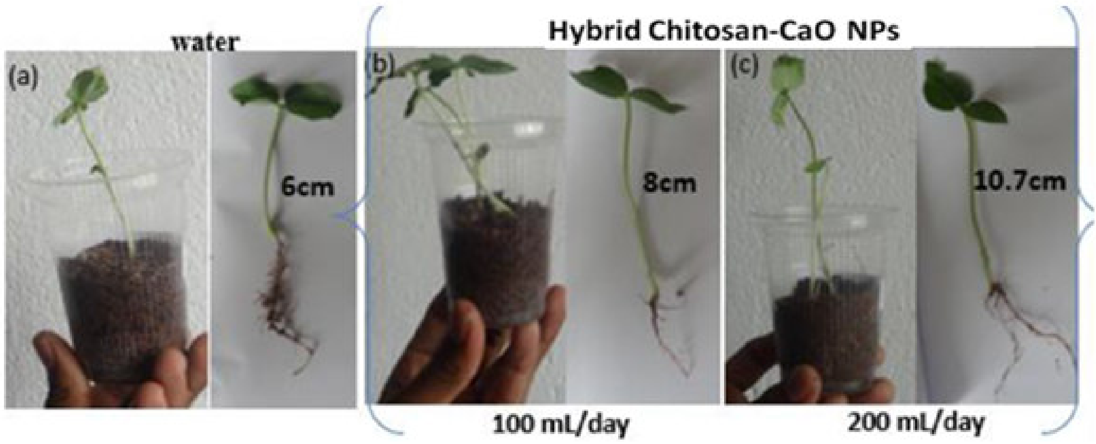


Figure 4

Black eye beans plant growth development after 7 days, (a) water treated seed, (b) 100mL/day hybrid chitosan-calcium oxide nanoparticles treated seed and (c) 200mL/day chitosan-calcium oxide nanoparticles treated seed

The present experiment shows the hybrid chitosan calcium oxide nanoparticles act nutrient transporter for plant growth development (Fig.4b and c). It revealed that at the end of the 7 days of experimental observation, the Black eye beans seed treated with hybrid chitosan-calcium oxide nanoparticles of 100ml/day showed a shoot length growth of about 8 cm and 200 ml/day treated Black eye beans seed showed a shoot length of 10.7 cm, whereas the well-watered Black eye beans seed (untreated) showed a shoot length growth of about only 6cm. Moreover, present study indicates that the hybrid chitosan-calcium oxide nanoparticles treated Black eye beans seed is growing faster as compared to untreated (water) Black eye beans seed. The growth of the plant is due to the interaction of the nanoparticles with soil microbial community and to sail microbes released nutrients to soil and they are responsible for the plant growth[20-22]. In the lab scale level after 7 days of experimental study, the hybrid chitosan-calcium oxide nanoparticles treated Black eye beans seed showed very good shoot growth. The present experiment explains the growth also depends on the concentration of hybrid chitosan-calcium oxide nanoparticles.

CONCLUSION

The hybrid chitosan-calcium oxide nanoparticle in plant growth development was confirmed for the first time which was an important development in agriculture sector. The prepared hybrid chitosan calcium oxide nanoparticles showed small grains of spherical shape. The XRD spectrum of indicated the polycrystalline nature of hybrid chitosan-calcium oxide nanoparticles with particles size varies from 1.97 to 8.94nm. The EDS spectrum revealed that the prepared sample is composed of Ca, O and N elements and the absence of any impurities. Moreover, present study revealed that the hybrid chitosan-calcium oxide nanoparticles treated Black eye bean plant is growing faster as compared to untreated Black eye bean plant in lab scale level. Thus application of chitosan nanoparticles composition in agriculture is promising and efficient nutrients to the desired plant parts, which is an utmost important outcome in agriculture development perspective.

CONFLICT OF INTEREST

Conflict of interest declared none.

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We sincerely thank the above reviewers for peer reviewing the manuscript