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RESEARCH ARTICLE

Green synthesis of calcium carbonate nanoparticles and their effects on seed germination and seedling growth of *Vigna mungo* (L.). Hepper.

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Abstract

The synthesis of nanoparticles has become the matter of great interest in recent times due to its various advantageous properties and applications in various fields. Though physical and chemical methods are more popular for nanoparticles (NPs) synthesis and the biosynthesis (green method) of nanoparticles using plant extracts is a better option due to its eco-friendliness. In this paper, we report the synthesis of $CaCO_3$ (Calcite) nanoparticles using $CaCl_2$ and selected plant species of *Boswellia ovalifoliolata* (an endemic plant) as the reducing agent. After exposing $CaCl_2$ to bark extract of plant, the nanoparticles are appeared in the form of precipitate. These are characterized by using Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray (EDAX) and Fourier Transform Infrared Spectroscopy (FTIR). The analysis of SEM information shows that the formation of stable nanoparticles are mostly spherical in shape with a diameter ranging from 40 to 75 nm and FTIR data revels that the plant extract containing phenolic compounds and proteins are act as reducing agents and the biologically synthesized NPs are calcite type of calcium carbonate nanoparticles (CCNP's). To observe the effect of CCNP's on seed germination and seedling growth of *Vigna mungo* shows that the bio-synthesized CCNPs accelerate the seed germination and seedling growth in *V. mungo* and shows the highest percentage of Seed germination, Seedling vigor index, Root and Shoot length, Fresh and Dry weight and Relative water content.

Introduction

Green synthesis of nanoparticles is an eco-friendly approach which might pave the way for researchers across the globe to explore the potential of different herbs in order to synthesize nanoparticles (Savithramma *et al*., 2011). For several years, scientists have constantly explored different synthetic methods to synthesize nanoparticles. On the contrary, the green method of synthesis of nanoparticles is easy, efficient, and eco-friendly in comparison to chemical mediated or microbe mediated synthesis. The chemical synthesis involves toxic solvents, high pressure, energy and high temperature conversion and microbe involved synthesis is not feasible industrially due to its lab maintenance. Since, green synthesis is the best option to opt for the synthesis of nanoparticles (Anamika *et al*., 2012) and biogenetic production is now of more interest due to simplicity of the procedures and versatility (Popescu *et al*., 2010).

Recently the different types of nanoparticles were bio-synthesized by using plant materials like Silver nanoparticles from *Shorea tumbuggaia*; *Svensonia hyderobadensis* (Ankanna and Savithramma, 2011; Linga rao and Savithramma, 2012), Gold nanoparticles from *Avena sativa* (Veronica *et al*., 2004), Gold, Silver & Silver-Gold alloys from *Azadirachta indica* (Shankar *et al*., 2004), Indium oxide nanoparticles from *Aloe vera* (Maensiri *et al* .,2008), Palladium nanoparticles from *Cinnamomum camphora* (Yang *et al*., 2009), Titanium-Nickel alloys from *Medicago sativa* (Schabes-Retchkiman *et al*., 2006), Iron oxide nanoparticles from *Medicago sativa* (Herrera-Becerra *et al*., 2008), Zinc oxide nanoparticles from *Sedum alfredii* (Qu *et al*., 2011), Copper nanoparticles from

Magnolia kobus (Lee *et al*., 2013), Cadmium Oxide Nanoparticles from *Achillea wilhelmsii* (Javad and Sasan, 2013).

Calcium is an alkaline material widely distributed in the earth. It is the fifth most abundant element (by mass), usually found in sedimentary rocks in the mineral forms of calcite, dolomite and gypsum. Plants need calcium for growth and development it activates number of enzyme activities, metabolisms, nitrate uptake (a useable form of nitrogen), biomass ratio (Savithramma, 2002) and photosynthetic rate (Savithramma, 2004; Savithramma *et al.*, 2007). It has been proved that the Ca^{2+} ameliorates the salinity and improved the plant growth. (Savithramma and Swamy, 1995; Kedarnath Reddy and Savithramma, 2013). Calcium is found in as many as 80 compounds sometimes called calcium salts such as calcium carbonate (lime). Calcium carbonate is a primary component of garden lime, also known as agricultural lime, which is used to enhance the soil quality by increasing P^H and water holding capacity of acidic soils. Calcium carbonate sources such as limestone and chalk, along with other chemical compounds are used in the preparation of agricultural lime, when added to the soil acts as a calcium source for plants. Calcium carbonate occurs in three main crystal polymorphs such as calcite, aragonite, and vaterite. Of these, calcite find in nature is more and is the most thermodynamically stable under ambient conditions (Sabriye, 2012).

The synthesis of calcium carbonate nanoparticles by different physical and chemical methods through Colloid particles (Sadowski *et al*., 2010), Protein cage (Hiroko *et al*., 2011), Modified emulsion membranes (Ritika *et al.*, 2004), Two membrane system (Zeshan *et al*., 2004), Saturated carbonate and Calcium nitrate aqueous solutions (Romuald *et al*., 2012) and by Ethanol assisted synthesis (Shao *et al*., 2013). In recent years, the development of efficient green methods for synthesis of metal nanoparticles has become a major focus. One of the most considered methods is production of metal nanoparticles by using plants (Ankanna *et al*., 2010). Even though several studies are concerned with the synthesis of CCNP's by using biological routes in micro organisms only by Long *et al* (2009). The green synthesis of CCNP's by plant material is not carried so far. Hence the present study was an attempt to made to synthesize the Calcium carbonate nanoparticles from *B. ovalifoliolata* and test the effect of synthesized CCNP's on seed germination and seedling growth of *V. mungo* (L.). Hepper.

Material and Methods

Selection of plant material:

Several plants like *B. ovalifoliolata, Shorea tumbbagia, Svensonia hyderobedensis* are screened for the synthesis of Silver nanoparticles (Savithramma, 2011). Among them *B. ovalifoliolata* are selected for the synthesis of CCNP's based on the formation of small size NP's when compare with other plants.

Boswellia ovalifoliolata

B. ovalifoliolata is a narrow endemic, endangered and threatened medicinal tree species collected in the year of 2012 from the Tirumala hills of Andhra Pradesh, India and were shade dried for 10 days then bark was kept in the hot air oven at 60^0C for 24 to 48 h. The dried bark was ground to a fine powder and used for biogenesis of CCNP's.

Synthesis of calcium carbonate nanoparticles:

The biological synthesis of calcium carbonate nanoparticles (CCNP's) was prepared by following the modified method of Long *et al* (2009).The plant extract was prepared by adding 10 gms of *B. ovalifoliolata* stem bark powder to 100 ml of distilled water. The mixture was continuously agitated up to 2 hours by using agitator. The extract was filtered and 25 ml were taken in 250 ml conical flask and add 50 ml of 5×10^{-2} mol L^{-1} $CaCl_2$ aqueous solution slowly and rinsed it. The P^H was maintained to 8.5 by using 1 mol L^{-1} ammonia aqueous solution and experiment are carried out in at the exposed conditions of air because CO_2 are used for synthesizing this NP's. The contents were stirred at 5000 rpm for 1 hour. These flasks were then plugged with cotton and incubated in at a room temperature of 27^0C for 2-3 days. The precipitate was appeared in the contents are separated by centrifugation. Washed three times with double-distilled water, ethanol, and chloroform respectively. The vacuum-dried powder was used further characterization.

SEM analysis:

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. The appeared precipitated powder was coated on the carbon coated copper grid and allowed to magnify the grid to know the shape and size of the particles.

EDAX measurements:

EDAX is an analytical technique used for the elemental analysis or chemical characterization of a sample. In order to carry out EDAX analysis the appeared powder was coated on carbon film and performed on Hitachi S-3400 N SEM instrument equipped with a Thermo EDAX attachment.

FTIR analysis:

To characterize the organic molecules mainly chemical bonds and molecular structures of bio-molecules responsible for the reduction of metal ions and their comparison with catalogued FTIR spectra enable to identify the material. The FTIR spectra were recorded in an FTIR spectrometer (BRUKER FTIR Tensor-27).

Growth parameters:

Vigna mungo

Seeds of Black gram (*Vigna mungo* L. Hepper cv. LBG-623) seeds were obtained from Regional Agriculture Research Centre, S.V. Agricultural College, Tirupati, Andhra Pradesh, India. The seeds were surface sterilized with 0.2 % $HgCl_2$ solution for 5 minutes with frequent shaking and washed thoroughly with distilled water. The seeds were presoaked in 50 ml of respected treatments up to 12 hours for examine the percentage of germination and seeds were shifted and germinated on fluted filter paper towels in bread boxes. The seedlings are now exposed to different treatments to examine the seedlings growth.

Seed viability test:

The seed viability test was assessed by performing tetrazolium (TZ) test as per the methods of Eplee and Norris (1987); Mitter (1993). The good quality seeds were separated from the bulk. 50 seeds were longitudinally bisected and seeds were incubated in 50 ml of 1% (w/v) solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC) prepared in 0.1 M Sorensen's buffer (pH 7.0) for 24 hours at 28 ^{0}C. After the incubation, seeds and the embryos were observed. The seeds where in embryos turned reddish pink were scored as viable and seeds that remained light yellow were scored as non-viable.

Percentage of Seed germination:

Seed germination (%) = (Number of germinated seeds/ Number of total seeds) ×100

Root and shoot length:

Root length was taken from the point below the hypocotyls to the end of the tip of the root. Shoot length was measured from the base of the root-hypocotyl transission zone up to the base of the cotyledons. The root and shoot length was measured with the help of a thread and scale.

Seedling vigour index:

The seedling vigour index was determined by using the formula given by Abdul baki and Anderson (1973). Seedling vigour index= Average root length in cms + Average shoot length in cms ×Germination percentage.

Fresh and Dry weight:

The fresh weight of root and shoot of seedlings was determined by weigh the root and shoot separately on electric balance. After the fresh weight taken then the seedlings was kept in a hot air oven at 80 ° C for 48 hrs then the weight of dry matter was recorded.

Determination of Relative water content (RWC)

RWC was calculated by using the method of Barrs and Weatherley, (1962). The seedlings were weighed and then dipped in distilled water for 4 h. The seedlings were then blotted dry and weighed prior to oven drying at 80 ^{0}C for 24 h.

$$\mathrm{RWC} = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Turgid weight} - \text{Dry weight})} \times 100$$

Results and Discussion

In the present study synthesis of calcium carbonate nanoparticles by using stem bark extract of *B. ovalifoliolata* and their effect on seedling growth of *V. mungo* was carried out. The *B. ovalifoliolata* stem bark extract having red colour at the time of extraction. When nanoparticle synthesis protocol was carried out the colour change was observed to brown followed by turbidity (Fig. 1). After 48 hours of incubation at 27±2^{0}c the precipitation was settled at the bottom of conical flasks. Which indicates the formation of NP's and analyzed by SEM, EDAX and FTIR. The SEM result shows that the nanoparticles are spherical in shape and having the size of 40-75 nm (Fig.2). The ammonia and CO_2 are responsible for the formation of calcite type of calcium carbonate nanoparticles. The same type of results was observed in the bacteria *Bacillus subtilis* (Long *et al*., 2009). The plant extract serves as a rich source of calcium ion, when reacted with CO_2 lead to formation of calcite crystals of highly irregular morphology indicating that bioorganic molecules present in extract modulate the crystals morphology (Sanayal *et al*., 2005). From EDAX spectrum, it is clear that *B. ovalifoliolata* has recorded the presence of metal calcium 22.12 weight percent and 14.64 atomic percent of nanoparticles (Fig.3, Table 1).

Fig 1: Colour change pattern of stem bark extract of *B. ovalifoliolata* from red to brown a) $CaCl_2$ b) Stem bark extract c) Synthesized CCNP's

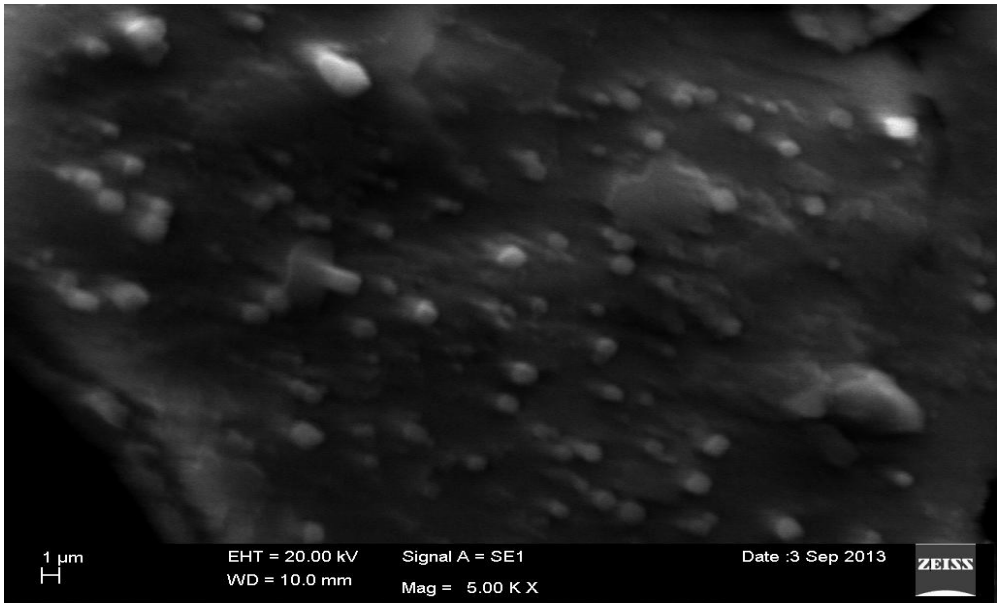


Fig 2: SEM analysis of calcite type of calcium carbonate nanoparticles having the size of 40-75 nms.

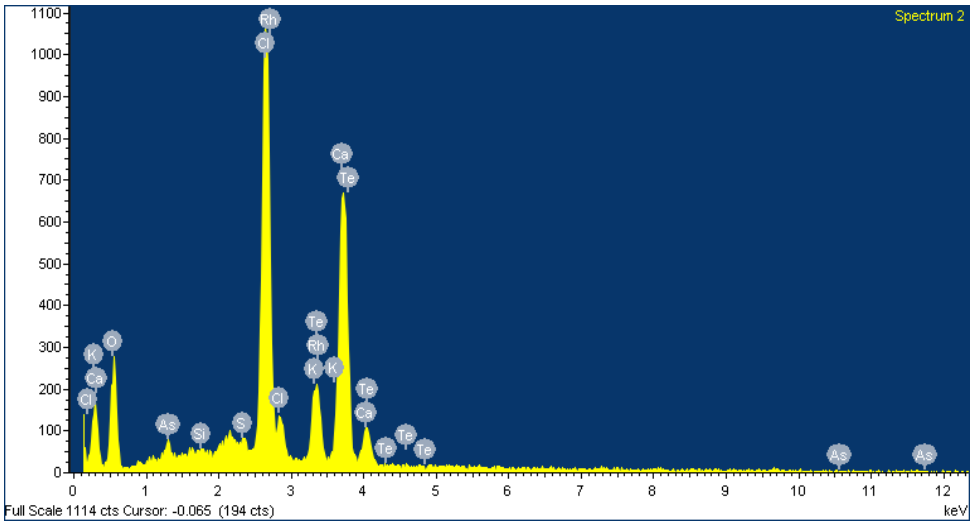


Fig 3: EDAX image of synthesized calcium carbonate nanoparticles

S. No	Element	Weight %	Atomic %
1.	O	36.35	60.27
2.	Si	0.16	0.15
3.	S	0.38	0.31
4.	Cl	23.85	17.85
5.	K	5.57	3.78
6.	Ca	22.13	14.64
7.	As	1.51	0.53
8.	Rh	7.50	1.93
9.	Te	2.55	0.53
10.	Totals	100.00	100.00

Table 1: Elemental analysis of EDAX

The analysis of FTIR data shows that the respective type of nanoparticles and what might be reason for synthesis of CCNP's. To carry out FTIR a spectrum showing molecular vibrations is obtained, in order to identify or characterize mainly organic materials. From such spectra information about the chemical bonds and molecular structure of a material can be obtained and their comparison with catalogued FTIR spectra enable to identify the material, *eg* the bio-molecules responsible for the reduction of NP's. The FTIR peaks are obtained at the 1455 cm^{-1}, 872 cm^{-1}, 712 cm^{-1} (Fig.4) shows that the synthesized naoparticles are calcite type of calcium carbonate nanoparticles. The previous literature on calcite shows that the proteins are responsible for the formation of calcite nanoparticles and FTIR data having the peaks which are indicate that they are calcite nanoparticles (Long *et al*., 2009) and showing the calcite is one of the forms of calcium carbonate. From the FTIR results it can be inferred that some of the bio-organics from stem bark extract of *B. ovalifoliolata* formed a strong reducing agent for nanoparticles (Fig. 5). Because the preliminary phytochemical screening of *B. ovalifoliolata* shows rich source of phenols and proteins in the stem bark and gum extracts (Savithramma *et al*., 2010). The FTIR spectra of phenol O-H stretch is at 3550-3200 cm^{-1} (IR chart). A typical FTIR spectrum of *B. ovalifoliolata* stem bark extract (Fig. 5) shows the several absorption peaks observed at 3443 cm^{-1}, 3112 cm^{-1}, 2958 cm^{-1}, 2914 cm^{-1}, 2855 cm^{-1}, 1747 cm^{-1}, 1612 cm^{-1}, 1591 cm^{-1}, 1515 cm^{-1}, 1489 cm^{-1}, 1448 cm^{-1}, 1418 cm^{-1}, 1377 cm^{-1}, 1344 cm^{-1}, 1288 cm^{-1}, 1224 cm^{-1}, 1169 cm^{-1}, 1098 cm^{-1}, 1068 cm^{-1}, 1047 cm^{-1}, 1011 cm^{-1}, 922 cm^{-1}, 861 cm^{-1}, 811 cm^{-1} 749 cm^{-1}, 690 cm^{-1}, 659 $cm^{-1}$and 615 cm^{-1}. The spectra at 3550-3200 cm^{-1} are the characteristic of hydroxyl functional group in alcohols and phenolic compounds. The bands at 2914 and 2855 cm^{-1} are could be due to alkane C–H stretch which is associated with lipid molecules. The IR band at 1747 cm^{-1} is stretch vibration of C=O, 1612 cm^{-1} is due to amide II bond from proteins (IR chart). The FTIR data of (Fig.5) shows that the stem bark extract having phenols and proteins but (in Fig. 4) the disappearance of phenols and proteins in synthesized nanoparticles. This typical FTIR data shows that the phenols and proteins are mainly act as reducing agents in the formation of calcite nanopartilces.

The biological molecules such as secondary metabolites could possibly play major role in the synthesis and stabilization of the nanoparticles (Inbakandan *et al*., 2010). Phenolic compounds are responsible for reduction of silver nanoparticles in *Dioscorea bulbifera* (Ghosh *et al*., 2012), silver and iron nanoparticles in *Sorghum (*Njagi *et al*., 2011), gold nanoparticles in *Justicia gendarussa* (Fazaludeena *et al*., 2012) and platinum nanoparticles in *Ocimum sanctum* (Soundarrajan *et* al., 2012). Naheed *et* al (2011) shows the release of proteins into solution by *Desmodium triflorum* and suggests a possible mechanism for the reduction of the metal ions. The synthesized platinum nanoparticles were characterized resulting in 2 to 12nm in size and FTIR analysis revealed that the formation of platinum nanoparticles was due to the biomolecules like proteins present in the leaf extract of *Diopyros kaki*. (Song *et al*., 2010). Proteins are responsible for reduction of silver nanoparticles in *Astragalus gummifer* (Kora *et al*., 2012), *Hydrilla verticilata* (Sable *et al*., 2012), *Piper betle* (Mallikarjuna *et al*., 2012) and palladium nanoparticles in *Glycine Max* (Petla *et al*., 2012). So, the FTIR spectra of present study are the indication of formation of calcite type of calcium carbonate nanoparticles and similar spectral data were studied by Long *et al* (2009); Shahraki *et al* (2011).

To observe the effect of synthesized CCNP's on seed germination and seedling growth of *V. mungo*. The sterilized seeds of uniform mass ratio are taken and presoaked in distilled water, 10 mM $CaCl_2$ and full strength concentration of synthesized CCNP' s (well sheared with double distilled water) of 50 ml each in 250ml conical flasks. After 12 h of presoaking seeds were germinated on fluted filter paper towels in bread boxes (five seeds per each box and calculations are taken three replicates) and record the percentage of germination. Seed germination and seedling emergence is most critical stage in seedling establishment and determining the successful crop production (Maghsoudi and Maghsoudi, 2008). After transformed the seedlings into bread boxes daily once given the treatment (5 ml) by distilled water as control, $CaCl_2$ as a standard (10mM $CaCl_2$ are standardized by Kedarnath Reddy and Savithramma, 2013) and synthesized calcium nanoparticles well sheared in double distilled water in each boxes respectively. Maintain the treated seedlings in well aerated and light conditions at 27 ± 2^0C room temperature. 6 days after treatment record the seedling vigor, root and shoot length, fresh and dry weight and relative water content.

The Tetrazolium test was carried in before the treatment and revealed 94% viability in the seeds and seed viability affecting many factors like i) when seeds reach maturity on the mother plant ii) they begin to deteriorate and the rate of deterioration and the rate of deterioration depend on the environmental conditions they experience. 92, 90 and 88 percentage of seed germination was recorded and 457, 689, 903 seedling vigor index (Table 2, graph 1) are recorded from control, 10 mM $CaCl_2$ and synthesized CCNP's respectively. The highest percentage of seed germination and seedling vigour were recorded from synthesized CCNP's. The root and shoot length of control, 10mM $CaCl_2$ and CCNP's treated seedlings are showed in Fig. 6; Table 2; Graph 2. The length in cms of seedlings is recorded in treated CCNP's, 10mM $CaCl_2$, and control in a decreasing order. The fresh and dry weight of root and shoot of control, 10mM $CaCl_2$ and CCNp's treated seedlings are showed in Table 2; Graph 3. The weight in gms of seedlings is recorded in treated CCNP's, 10mM $CaCl_2$, control in an decreasing order. The relative water content of seedlings was decreased in an order of CCNP's, 10mM $CaCl_2$ and control (Table 2; Graph 4). The overall effect of synthesized NP's shows the positive effects on seed germination and seedling growth of *V. mungo*.

The positive effects on germination of aged spinach seeds and on the growth of seedlings were obtained if the seeds were soaked in high-strength TiO_2-nanoparticles-solution (0.25 to 4%) and the best results provided application of 2500 mg/dm^3 nano-TiO_2. The TiO_2 NPs were found to promote growth of spinach and accelerate nitrogen assimilation (Yang *et al*., 2006). Sheykhbaglou *et al* (2010) tested the effects of nano-iron oxide particles applied in the form of spray on agronomic traits of soybean in field experiments and found that nano-iron oxide at the concentration of 0.75 g/dm^3 increased leaf + pod dry weight and pod dry weight. Application of 0.5 g/dm^3 nano-iron oxide particles resulted in the highest grain yield showing 48% increase in comparison with control. Nano-iron oxide was also found to facilitate the photosynthate and iron transferring to the leaves of peanut**. (**Liu., *et al* 2005**).** Analysis of the influence of magnetic nanoparticles coated with tetra methyl ammonium hydroxide on the growth of *Zea mays* plant in early ontogenetic stages showed that application of small ferrofluid concentrations (10-50 mm^3/dm^3) induced plant length stimulation, the increase of chlorophyll *a* **(**up to 13%) as well that the nucleic acid level (up to 10%) in maize plantlets during their first days of life (Racuciu and Creanga**,** 2007). Application of nanoparticles such as silica, palladium, gold and copper nanoparticles significantly influenced the growth of lettuce plants after 15 days of incubation which was reflected in an increase in the shoot/root ratio compared to that of the control. (Shah and Belozerova, 2009). Sprouting of *B. ovalifoliolata* seeds treated with SNP's was observed from 7th day onwards.. Whereas, the sprouting was observed on 15th day in control seeds. All seeds treated with SNPs completed the germination with in 7 to 10 days. However 10 to 20 days required for control seeds. The seedlings grown in MS media supplemented with SNPs increased length when compare to the control seedlings (Savithramma *et al*., 2012).

Cells of plants, algae, and fungi possess cell walls that constitute a primary site for interaction and a barrier for the entrance of NPs. Inside the cells NPs might directly provoke alterations of membranes and other cell structures and molecules, as well as protective mechanisms (Navarro *et al*., 2008). Calcium treatment caused an increase in the shoot and root length during the seedling growth. Plants and animals absorb calcium carbonate from water - where it exists, in most cases, in the dissolved form of calcium hydrogen carbonate Ca $(HCO_3)_2$ - and use it to build up their skeletons and shells. Using soluble calcium to stimulate plant growth (Sam and Lloyd, 1914). Sufficient concentrations of calcium increases ammonium, potassium and phosphorus absorption, stimulates photosynthesis, and increases the size of plant parts. It also makes the use of nitrogen more efficient, which improves the economics of production and reduces nitrogen contamination of the environment. Early studies on the role of Ca^{2+} in the growth noted that low concentration led to the reduction of cell division in the roots (Jones and Lunt, 1967). The target for low Ca^{2+} action might include the formation of cell plate and mitotic apparatus. (Marcum *et al*., 1978) as plant cell spindles contained Calmodulin (Hepler and Wayne, 1985). Ca^{2+} and Calmodulin involved in the cell plate formation and assembly and disassembly of microtubules.

Ca^{2+} is an essential element; however, its role is elusive. When examining total Ca^{2+} in plants, the concentration is quite large (mM), but its requirement is that of a micronutrient (mM). Ca^{2+} is not usually limiting in field conditions, still there are several defects that can be associated with low levels of this ion, including poor root development, leaf necrosis and curling, blossom end rot, bitter pit, fruit cracking, poor fruit storage, and water soaking (Simon, 1978; White and Broadley, 2003). Calcium chloride ameliorates salt stress in *V. mungo* and promotes the growth and biochemical changes in seedlings of *V. mungo* (Kedarnath Reddy and Savithramma, 2013) and postpone the senescence of primary leaves in *V. unguiculata* (Savithramma and Swamy, 1989) $CaCO_3$ is one of the rich source of calcium and is supplemented in the form of garden lime. Several studies are concerned with the synthesis of nanomaterials using biological routes and promotory effects on seedling growth. So far no studies have been reported on the biological synthesis and promontory effects of calcium carbonate nanoparticles on plants. Hence the present study was undertaken to synthesize CCNP's and know the effect on seed germination and seedling growth of *V. mungo*. The results revealed that the stem bark extract of *B. ovalifoliolata* is the best source for biological synthesis of CCNP' s and accelerate the seed germination and seedling growth of *V. mungo* when compare with calcium chloride salt solution.

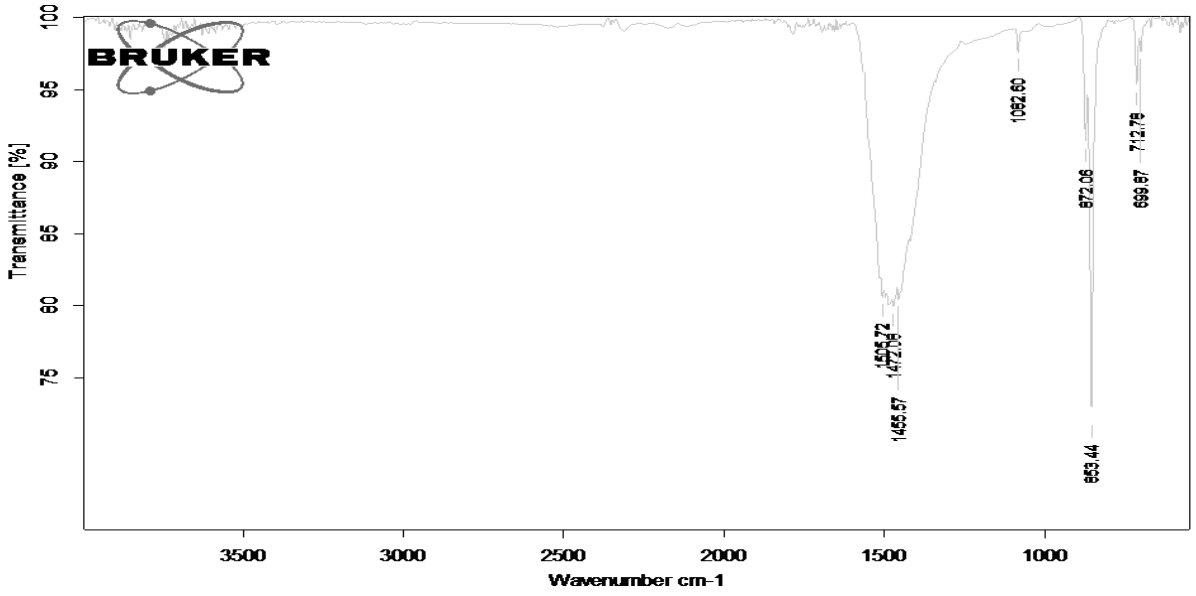


Fig 4: FTIR spectra of synthesized CCNP's shows the presence of calcite and disappearance of phenols and proteins.

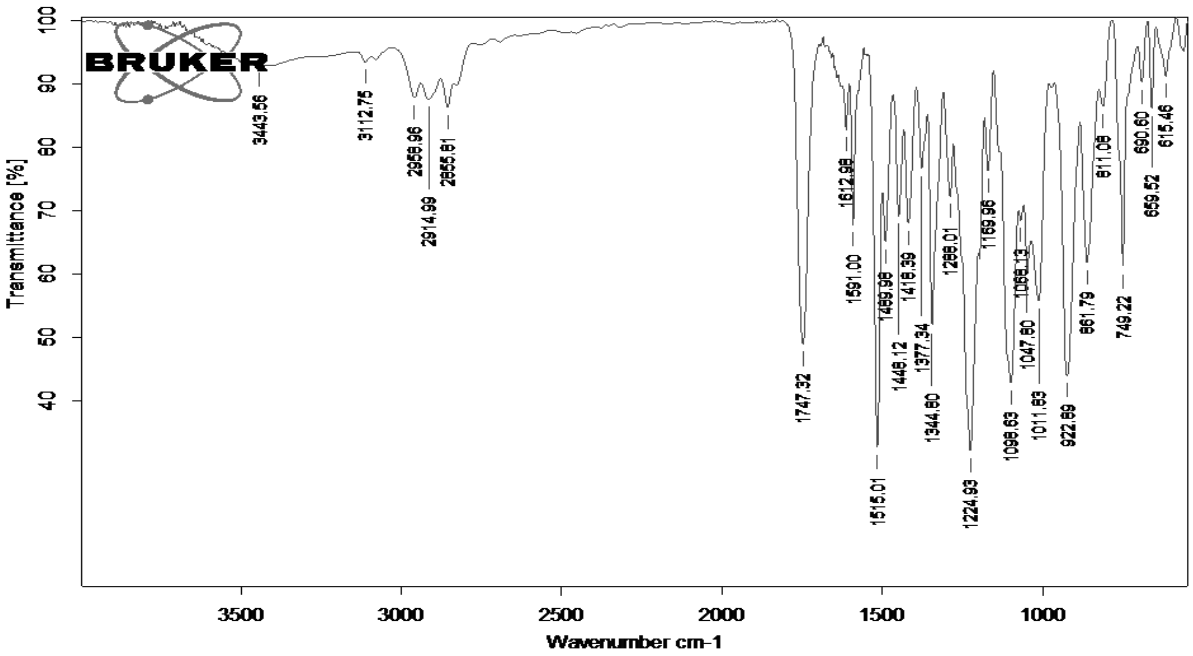


Fig 5: FTIR spectra of *B. ovalifoliolata* stem bark extract shows the presence phenols and proteins.

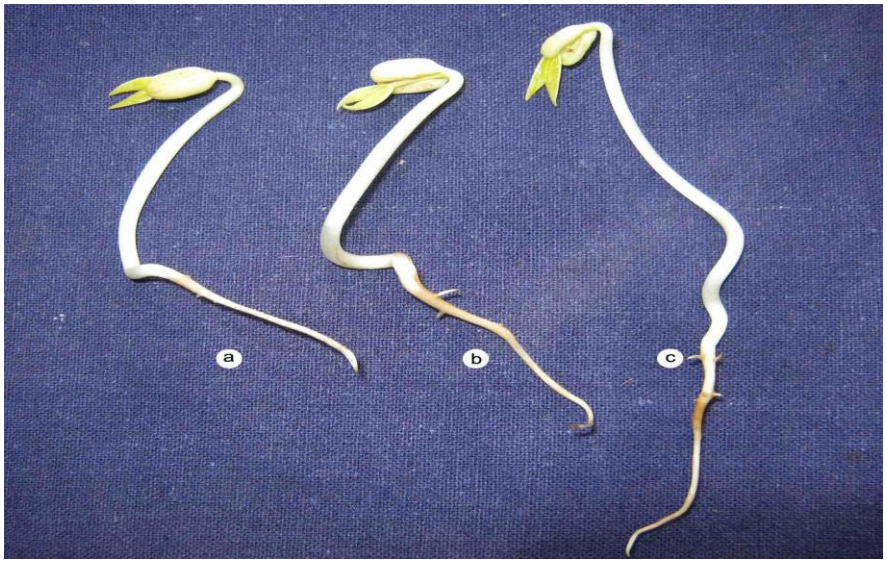
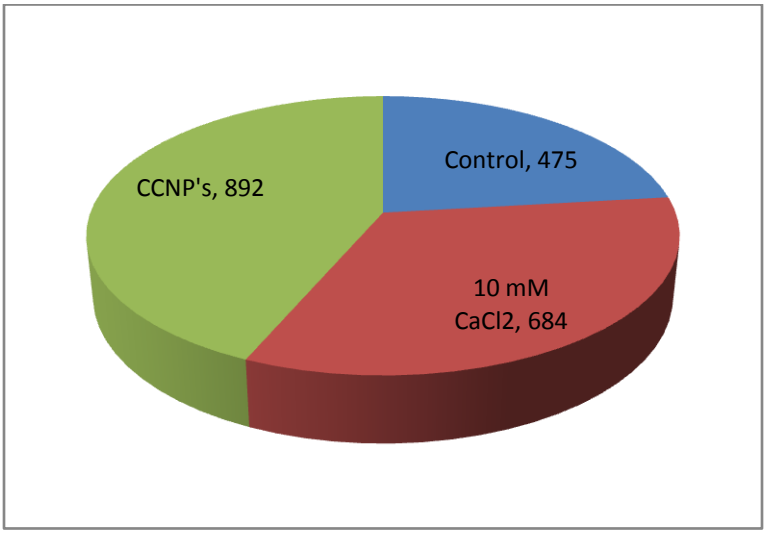


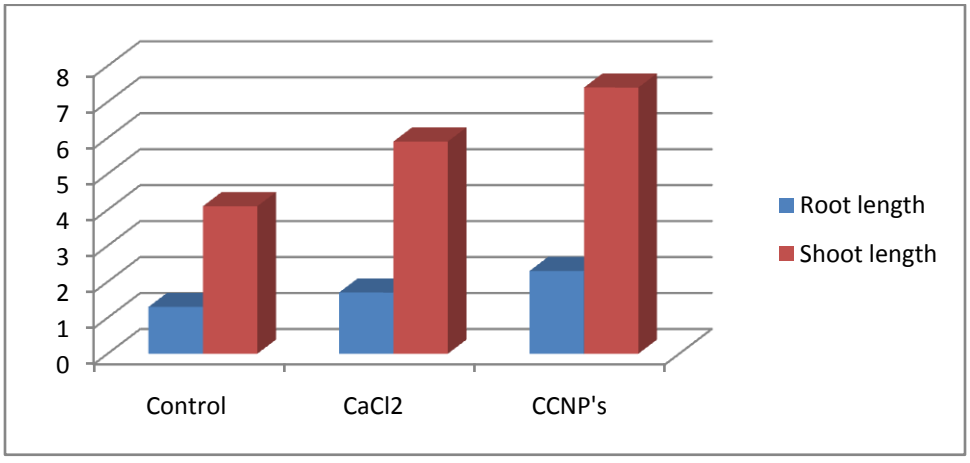
Fig 6: a) control b) 10mM $CaCl_2$ c) CCNP's treated seedling growth in *V. mungo*

Treatments	Seed germi-nation (%)	Seedling Vigor Index	Seedling growth (cms)		Fresh Weight (gms)		Dry weight (gms)		Relative Water Content (%)	
			Root length	Shoot length	Root	Shoot	Root	Shoot	Root	Shoot
Control	88	475	1.3 ± 0.070	4.1 ± 0.511	0.043 ± 0.004	0.481± 0.070	0.0062 ± 0.0007	0.116 ± 0.0024	77.40 ± 3.61	82.02 ± 3.36
10 mM $CaCl_2$	90	684	1.7 ± 0.141	5.9 ± 1.127	0.086 ± 0.004	0.583± 0.672	0.0074 ± 0.0007	0.121 ± 0.0141	79.34 ± 1.11	87.05 ± 0.99
CCNp's	92	892	2.3 ± 0.216	7.4 ± 0.108	0.096 ± 0.008	0.653± 0.024	0.0081 ± 0.0966	0.131 ± 0.0007	83.21 ± 0.44	89.98 ± 0.75

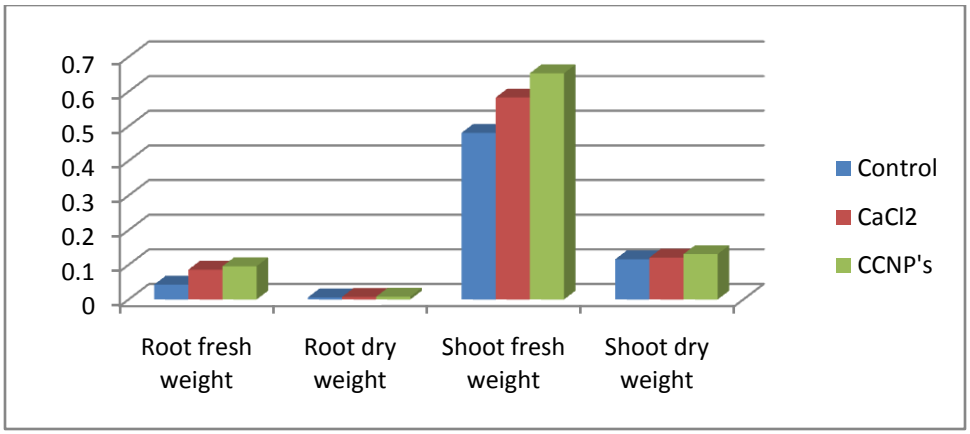
Table 2: Effect of control, 10mM $CaCl_2$ and CCNP's on seed germination, Seedling vigor index, growth, fresh, dry weight and relative water content of *V. mungo*. Values indicates an average of three samples ± S. E



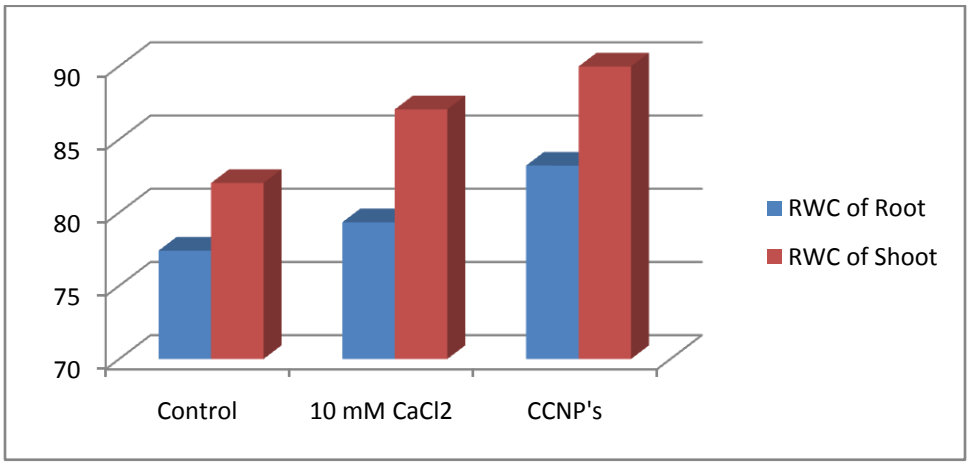
Graph 1: Seedling vigor index of respected treatments



Graph 2: Root and shoot length of respected treatments



Graph 3: Fresh and dry weight of root and shoot of respected treatments



Graph 4: Relative water content of respected treatments

Conclusion

In recent times Calcim carbonate nanoprtciles are synthesized rapidly in physical and chemical methods. Nowadays the biological synthesis of nanoparticles is an important branch of nanotechnology. The present study concluded that the CCNP's are synthesized also in biological friendly pathway and they have positive growth promontory effects on growth of *V. mungo* seedlings. The synthesized nanoparticles under lab conditions are observed that they play an important role in seed germination and seedling growth of *V. mungo*. But field experiments are required to analyze the effect of nanoparticles on crop plants and is important to sustainable greater yield of crops. Further studies are required to synthesize the nanoparticles in reduced size and they having any microbial activities. Because most of the micro organisms are sensitive to nanoparticles.

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