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**Original Research Article**

# Facile and Eco-Friendly Method for Synthesis of Calcium Oxide (CaO) Nanoparticles and its Potential Application in Agriculture

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## Abstract

The regular techniques used to incorporate organic and inorganic compounds are experiencing significant expense, not environmental friendly, low proficient and not progressively reasonable in huge scope activities. As of late, we need a productive and eco-friendly manufactured way to deal with blend some significant inorganic or organic compounds in nano scales. Green procedures have the minimization of hurtful synthetic compounds and instrumentations, ease, basic, no unsafe concoction ages and high proficiency. In nano-scale, the capability of the compounds altogether increments and the green synthesis methodologies in engineered science are better other options and effective over customary techniques. In the current investigation, we have synthesized calcium oxide (CaO) nanoparticles by utilizing the leaf extract of *Ocimum tenuiflorum*. Calcium oxide nanoparticles are discovered important in adsorption, antimicrobial activities, catalysis and absorption. The newly synthesized CaO nanoparticles (CaONP) have been described by various systematic techniques, for example, UV-Visible, DLS, XRD, SEM and EDX. The CaO nanoparticles are pertinent in the antimicrobial activity against gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) and huge zone of inhibition found over these microorganisms in the following order, *S. aureus* < *B. subtilis* < *E. coli* < *P. aeruginosa*. CaONP also test for agricultural application as macro nutrient. Plant growth was measured using growth analysis parameters i.e. % germination, relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), leaf weight ratio (LWR), specific leaf area (SLA), specific leaf weight (SLW), leaf area duration (LAD), the physiological response to particular mineral stress calculated by measuring % phytotoxicity, % inhibition, tolerance indices, seed vigor index. The biochemical response to particular CaNP stress calculated by measuring total carbohydrates, total protein, chlorophyll pigment concentrations and peroxidase enzyme activity.

**Keywords:** Green synthesis, leaf extract of *Ocimum tenuifloru*, Anti-microbial activity, potential application in agriculture, CaO nanoparticles.



## INTRODUCTION

The synthesis of nanoparticles has become the matter of extraordinary enthusiasm for ongoing occasions because of its different beneficial properties and applications in different fields. In spite of the fact that physical and chemical techniques are increasingly well known for nanoparticles (NPs) synthesis and the biosynthesis (green strategy) for nanoparticles utilizing various plant extracts is a superior choice because of its eco-invitingness. Green synthesis of nanoparticles is an eco-friendly methodology which may prepare for scientists over the globe to investigate the capability of various spices so as to synthesis nanoparticles (Savithramma *et al*., 2016). For quite a long while, researchers have continually investigated distinctive engineered techniques to synthesis nanoparticles. In actuality, the green technique for synthesis of nanoparticles is simple, effective, and environmental friendly in contrast with physical, chemical or microorganism mediated synthesis. The chemical synthesis includes harmful solvents, high weight, vitality and high temperature transformation and organism included combination isn't plausible modernly because of its lab support. Since, green synthesis is the most ideal alternative to decide on the synthesis of nanoparticles (Anamika *et al*., 2012) and biogenetic production is presently of more enthusiasm because of

straightforwardness of the systems and adaptability (Popescu *et al*., 2010).

Recently the different types of nanopaticles were synthesized by using plant materials like silver nanoparticles from Ficus elastica leaf extract (Gandhi *et al*., 2014), leaf extract of Clitoria ternatea and Solanum nigrum (Krithiga *et al*., 2015). Gold nanoparticles from Avena sativa (Veronica *et al*., 2004). Copper nanoparticles from the seed extracts of Piper nigrum (Gandhi *et al*., 2018), Sesamum indicum (Gandhi *et al*., 2014). Calcium nanoparticles by using Ocimum sanctum leaf extract (Vijay *et al*., 2020), Rhododendran arboretum leaf extract (Bharti *et al*., 2019), Yugandhar and savitramma synthesized calcium carbonate nanoparticles by using Boswellia ovalifoliolata (Yugandhar & Savitramma 2013).

Calcium is a basic material broadly conveyed in the earth. It is the fifth most bountiful element (by mass), typically found in sedimentary rocks in the mineral types of calcite, dolomite and gypsum. Plants need calcium for development and improvement it enacts number of compound exercises, activates various types of enzymes as a co factor, participates membrane transport metabolisms, nitrate take-up (a useable type of nitrogen), biomass proportion (Savithramma, 2002) and photosynthetic rate (Savithramma, 2004; Savithramma *et al*., 2007). It has been demonstrated that the $Ca^{2+}$ enhances the saltiness and improved the plant development (Savithramma & Swamy, 1995; Kedarnath Reddy & Savithramma, 2013). Calcium is found in upwards of 80 compounds some of the time called calcium salts, for example, calcium carbonate (lime). Calcium carbonate is an essential part of nursery lime, otherwise called agrarian lime, which is utilized to improve the dirt quality by expanding pH and water holding limit of acidic soils. Calcium carbonate sources, for example, limestone and chalk, alongside other synthetic compounds are utilized in the readiness of agrarian lime, when added to the dirt goes about as a calcium hotspot for plants. Calcium carbonate happens in three primary precious stone polymorphs, for example, calcite, aragonite, and vaterite. Of these, calcite find in nature is more and is the most thermodynamically stable under surrounding conditions (Sabriye, 2012).

The synthesis of calcium and calcium oxide 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 Ca/CaO nanoparticles by using biological routes in micro organisms only by Long *et al*., 2009. The green synthesis of Ca/CaO nanoparticles by plant material is not carried so far. Hence, the present study was an attempt to synthesize the CaO nanoparticles from leaf extract of Ocimum tenuiflorum and test the effect of synthesized of CaO nanoparticles on seed germination and seedling growth of Cicer arietinum at laboratory conditions. The schematic work protocol illustrated in figure-1.

## MATERIALS & METHODS

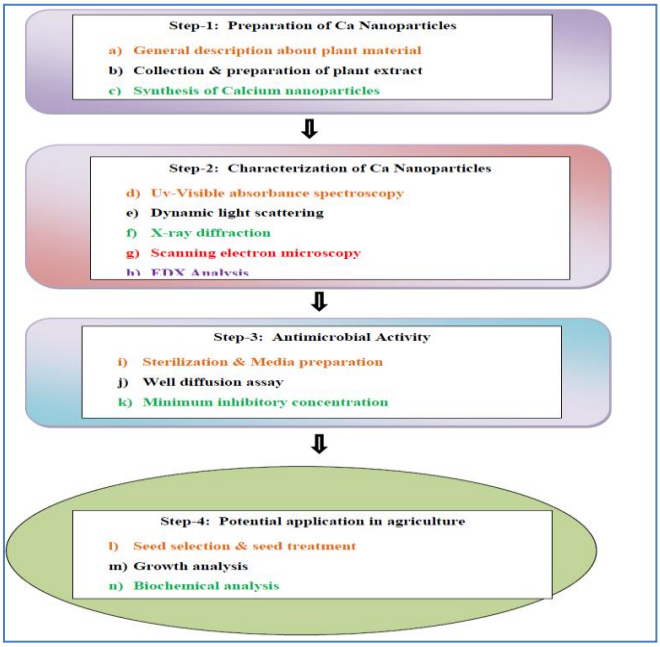


**Fig-1: Schematic flow chart of methodology of present study**

**Step-1: Phytosynthesis of Ca nanoparticles**

**General Description about plant material**

Ocimum tenuiflorum (Figure-2) is firmly identified with culinary basil (Ocimum basilicum), however contrasts in being a fleeting perpetual with littler blossoms. Ordinarily known as holy basil or tulsi and tulasi in South Asia, it is a significant holy plant in Hinduism and, similarly as with many plant species utilized in Asia, the strict uses are regularly connected with the restorative employments. Verifiably, holy basil was every now and again developed in huge vessels in the patios of Hindu posts and sanctuaries to purge the body. One of the plant's equivalent words, Ocimum sanctum, mirrors this strict association. These are lectotype aromatic herbs or woody herbs. Stem is erect, branched and woody at base. It contains patent hairs. Leaves petiolate, blade broadly elliptical, 1.5–3.3 × 1.1–2 cm, serrate, apex obtuse, base cuneate, with an indumentum of short, appressed hairs, petiole 7–15 mm long. The biological classification of Ocimum tenuiflorum is given below.


**Fig-2: Classification and morphological appearance of Ocimum tenuiflorum**

**Collection of plant material & preparation of plant extract**

Fresh leaves of Ocimum tenuiflorum, free from diseases were collected from botanical garden of green fields institute of agriculture research and training, located at Ibrahimpatnam, Rangareddy, Telangana and then washed thoroughly 2-3 times with tap water and once with sterile water. 20 g of fresh leaves was finely chopped and added to 100 mL of distilled water and stirred at 60°C for 1 h. After boiling, the mixture was cooled and filtered with Whatman paper number 1. Filtrate was collected.

**Synthesis of calcium oxide (CaO) nanoparticles**

Synthesis of Calcium Nanoparticles was carried by 95 mL of 0.1 M of aqueous solution of calcium chloride ($CaCl_2$) and 5 mL of leaf extract of Ocimum tenuiflorum, for bio reduction process at room temperature.

**Step-2: Characterization of phytosynthesized CaO nanoparticles**

**UV-Visible absorbance spectroscopy**

UV-Visible spectroscopy analysis was carried out on a Systronic UV-Visible absorption spectrophotometer with a resolution of ±1 nm between 200 and 1000 nm processing a scanning speed of 200 nm/min. Equal amounts of the suspension (0.5 mL) were taken and analyzed at room temperature. The progress of the reaction between calcium ions and the leaf extract was monitored by UV-Visible spectra of calcium nanoparticles in aqueous solution with different wavelength in nanometers from 340 to 800 nm. The reduction of calcium ions and formation of calcium nanoparticles occurred within a hour of reaction. Control was maintained by using calcium chloride solution ($CaCl_2$).

**Particle size and distribution**

The size distribution of CaO nanoparticles was determined by means of dynamic light scattering using a photon correlation spectrophotometer equipped with single frequency laser sources (HORIBA Scientific SZ-100). The intensity of scattered light was detected at the angle of 173° to avoid overestimation arising from the scattering of larger particles, the hydrodynamic diameter (dh) was obtained as a value at peak maximum of the size volume distribution function. Two modes of measurement were used: a) time-averaged measurements, in which measurements were average of 12 runs each lasting around 10 s, and b) time-resolved measurements in which the results were obtained from a single measurement lasting about 11s. Each sample was measured five times, and representative data are shown. All measurements were conducted at 25.0 ± 0.1 °C.

**X-ray diffraction analysis**

X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. It is a technique used for determining the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of incident X-rays to diffract into many specific directions. A thin

film of the silver nanoparticle was made by dipping a glass plate in a solution and carried out for X-ray diffraction studies. The crystalline silver nanoparticle was calculated from the width of the XRD peaks and the average size of the nanoparticles can be estimated using the Debye-Scherrer equation.

$$D = \frac{K\lambda}{\beta \, Cos \, \theta}$$

Where K is instrument constant (0.9), λ is wavelength of X ray diffraction (0.1541 nm) 'β' is FWHM (full width at half maximum), 'θ' is the diffraction angle and 'D' is particle diameter size.

**Scanning electron microscopy & EDX analysis**

In the present investigation, Jeol JSM-6480 LV SEM machine was used to characterize mean particle size, morphology of nanoparticles. The phytosynthesized CaO nanoparticles powder sample was sonicated with distilled water, small drop of this sample was placed on glass slide allowed to dry. A thin layer of platinum was coated to make the samples conductive Jeol JSM-6480 LV SEM machine was operated at a vacuum of the order of 10-5 torr. The accelerating voltage of the microscope was kept in the range 10-20 kV. Compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached with the SEM. The EDX analysis of CaO sample was done by the SEM (JEOL-JSM 5800) machine. The EDX normally reveals the presence of phases.

**Step-3: Anti-microbial activity of CaO nanoparticles**

**Microorganisms**

Representative microorganisms of Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus) and Gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli) were used to evaluate the antimicrobial activity of prepared CaO nanoparticles & microbial cultures were collected from school of life sciences, Jawaharlal Nehru institute of advanced studies, Hyderabad. Bacterial strains were maintained on nutrient agar slants at 4 $^{o}$C.

Two tests were carried out to assess the antibacterial activity of CaO nanoparticles. The first test was to identify the minimum inhibitory concentration (MIC) of the synthesized CaO nanoparticles. The second test consisted of agar well diffusion method where the zone of inhibition determines the extent of antibacterial activity for all the three solutions ($CaCl_2$ aqueous solution, plant extract and $CaCl_2$ + plant extract) against gram positive and gram negative bacteria.

**Minimum inhibitory concentration**

The MIC of CaO nanoparticles was identified to determine the lowest concentration that inhibits the visible growth of the test organisms. Suspensions of the test organism were swabbed on the culture medium. Wells were then carved on the plates. Different concentrations of the CaO nanoparticles (5, 10, 15, and 20 µg/mL) were added to the wells. All the plates were incubated at 37°C for 24 hours in order to determine the inhibitory growth of the CaO nanoparticles on particular bacterial organism. The procedure was repeated three times and the mean value was taken into consideration.

**Well diffusion assay**

The antibacterial activity of the phyto synthesized CaO nanoparticles were determined by following the agar plate well diffusion method. 15 agar plates were sterilized and 12 plates were inoculated with respective bacterial cultures i.e. gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus) and gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli) which were prepared freshly. 2 plates were kept for positive control and one plate left for negative control. With the help of a sterile stainless steel cork borer, four agar wells of 6 mm diameter each were contrived in all the agar plates used. The wells were labeled as Group A, B, C, and D. The wells A and B were loaded with 20 µL of the $CaCl_2$ aqueous solution and leaf extract of Ocimum tenuiflorum (15 µg/mL), respectively. The well C was loaded with 20 µg/mL of the ($CaCl_2$ aqueous solution and leaf extract of Ocimum tenuiflorum) combination. The well D was loaded with 20 µg/mL of streptomycin which was used as the positive control. The plates were incubated at 37°C for 24 h and the zone of inhibition (ZOI; mm) that appeared around the wells was recorded. The values were tabulated using Microsoft Office Excel 2007 and subjected to statistical analysis using the Graphpad prism software version 6.0. One-way ANOVA test was used to compare within the groups and between groups. The level of significance was set at 5%.

**Step-4: Potential Application of phytosynthesized CaO nanopaticles in agriculture**

Present experiments were conducted for 30 days (from 05/01/2020 to 04/02/2020) at Green Fields Institute of Agriculture Research & training, Ibrahimpatnam, Rangareddy, Telangana, India, to evaluate the impact of phytosynthesized CaO nanoparticles on Bengal gram (Cicer arietinum). For this the soil samples were collected from open fields located at research institute during the season of december-2019. The collected soil samples dried under sunlight for four days and cleaned by removing all vegetation & solid unwanted materials. Then the samples were transferred into plastic tubes for further experiments. The physico chemical analysis of soil and water used for present study carried by standard protocols of APHA 2$^{nd}$ edition. The calculated values represented in Table-1 (water quality parameters) & Table- 2 (soil quality parameters).

Into a series of 12 plastic tubs equal quantity of soil (≈ 2 kg/tub) was transferred and tubs were denoted as C (control), T1 (lower concentration), T2 (medium concentration) and T3 (higher concentration) and CT (seeds presoaked in 10 mM $CaCl_2$ solution). All the experiments carried out in triplets and mean values were taken for results analysis.

**Seed Selection & Seed Treatment**

The certified and pretreated seeds of Bengal gram (Cicer arietinum) used in current investigation were purchased from local market located at Ibrahimpatnam, rangareddy district, Telangana, India. Hence, there is no further pretreatment performed before sowing the seeds into the experimental tubs.

The set of 30 seeds were presoaked into solution-A full strength concentration of synthesized CaO nanoparticles (well sheared with double distilled water) 50 ml and made upto 250 ml with distilled water and denoted as T1 treatment. The second set of 30 seeds were soaked in solution-B, which prepared by using 100 ml CaO nanoparticle solution and made upto 250 ml with double distilled water & was denoted as T2 treatment. The third set of 30 seeds were soaked in solution-C, which prepared by using 150 ml CaO nanoparticle solution and made upto 250 ml with double distilled water & was denoted as T3 treatment. A set of 30 seeds were sown into normal/ untreated soil and considered as control. Another set of 30 seeds were presoaked into 250 ml of 10 mM $CaCl_2$ solution for 12 hours and denoted as calcium chloride treatment (CT). The pre soaked T1, T2, T3 and CT Bengal gram (Cicer arietinum) seeds were sown into respective tubs randomly and irrigated with bore well water immediately. From the time of seed sowing the experimental tubs were irrigated regularly once in a day to maintain soil moisture at saturated level. The experimental setup kept in open for better sunlight and air.

**Growth Analysis**

The following data are required to calculate different growth parameters in order to express the instantaneous values and mean values over time interval. In the following discussion W, WL, WS and WR are used to represent the dry weights of total plant, dry leaves, stem and roots respectively. Whereas A is the leaf area.

**Relative Growth Rate (RGR)**

The term RGR was coined by Blackman. It is defined as the rate of increase in dry matter per unit of dry matter already present. This is also referred as Efficiency index, since the rate of growth is expressed as the rate of interest on the capital. It provides a valuable overall index of plant growth. RGR can be calculating by following formulae.

$$\text{Relative Growth Rate} = \frac{\log e^{W_2} - \log e^{W_1}}{T_2 - T_1}$$

**Net Assimilation Rate (NAR)**

The NAR is a measure of the amount of photosynthetic product going into plant material i.e. it is estimate of net photosynthetic carbon assimilated by photosynthesis minus the carbon lost by respiration. The NAR can be determined by measuring plant dry weight and leaf area periodically during growth and is commonly reported as grams of dry weight increase per square centimeter of leaf surface per a particular time period. This is also called as unit leaf rate because the assimilatory area includes only the active leaf area in measuring the rate of dry matter production. The mean NAR over a time interval from $T_1$ to $T_2$ is given by

$$\text{NAR} = \frac{W_2 - W_1}{T_2 - T_1} X \frac{\log e^{A_2} - \log e^{A_1}}{A_2 - A_1}$$

**Leaf Area Ratio (LAR)**

The LAR is a measure of the proportion of the plant which is engaged in photosynthetic process. It gives the relative size of the assimilatory apparatus. It is also called as capacity factor. It is defined as the ratio between leaf area in square centimeters and total plant dry weight. It represents leafiness character of crop plants on area basis.

$$\text{Leaf Area Ratio} = \frac{A}{W}$$

**Leaf Weight Ratio (LWR)**

It is one of the components of LAR and is defined as the ratio between grams of dry matter in leaves and total dry matter in plants. Since the numerator and denominator are on dry weight basis LWR is dimensionless. It is the index of leafiness of the plant on weight basis.

$$\text{Leaf Weight Ratio (LWR)} = \frac{W_L}{W}$$

**Specific Leaf Area (SLA)**

It is another component of LAR and defined as the ratio between leaf area in $cm^2$ and total leaf dry weight in grams. This is used as a measure of leaf density. The mean SLA can be calculated as follows

$$\text{Specific Leaf Area (SLA)} = \frac{A}{W_L}$$

**Specific Leaf Weight (SLW)**

The reciprocal of SLA is called SLW. It is defined as the ratio between total dry weight and leaf area. It indicates the relative thickness of the leaf of different genotypes.

$$\text{SpecificLeafWeight(SLW)} = \frac{W_L}{A}$$

**Leaf Area Duration (LAD)**

It is usually expressed as a measure of leaf area integrated over a time period. Some takes into account both the magnitude of leaf area and its persistence in time. It also represents the leafiness of the crop growing period. Thus the unit of measurement of LAD may be in day or weeks or months.

$$\text{LeafAreaDuration(LAD)} = \frac{LA_1 + LA_2\,(T_2 - T_1)}{2}$$

**Plant Sampling and Analysis**

A seed was considered as germinated when root had emerged more than 2 mm. The number of germinated seeds per time is termed as seed germination rate. Germination percentage and tolerance indices determined by the following formula (Iqbal & Rahmati, 1992).

$$\%\ \text{ofGermination} = \frac{\text{NumberofSeedsGerminated}}{\text{TotalNumberofSeedsPlanted}} X\ 100$$

$$\text{Toleranceindices} = \frac{\text{Meanrootlengthoftreatedseed}}{\text{Meanrootlengthofcontrol}}$$

The inhibition of seedling growth was expressed according to the formula (Chou & Muller, 1972).

$$\text{percentageofinhibition} = \frac{\text{Lengthofcontrol} - \text{Lengthoftreatedseed}}{\text{Lenghtofcontrol}} X\ 100$$

**Seedling Vigor Index**

Seedling vigor index are those properties of the seed which determine the levels of activity and performance of the seed during germination and seedling emergence. It is a single measurable property like germination describing several characteristics associated with various aspects of the performance of seed. Seedling vigor index is calculated by using formula (Absul baki & Anderson, 1993; Bewly & Black, 1982).

SVI = Germination percentage × Seedling length

**Percentage Phyto-toxicity**

Percentage phytotoxicity of heavy metals on root and shoot growth of pegion pea (Cajanus cajan) were calculated at regular time interval (5 to 30 days of seedling growth). The following formula was used for calculating the percentage phytotoxicity (Gang *et al*., 2013).

$$\text{PercentageofPhytotoxicity} = \frac{\frac{S}{R}\text{lengthofcontrol} - \frac{S}{R}\text{lengthoftreatedseed}}{\frac{S}{R}\text{lengthofcontrol}} X\ 100$$

**Estimation of biochemical attributes**

Biochemical attributes were studied in term of photosynthetic pigments. The chlorophyll-a, chlorophyll-b and total chlorophyll (a + b) were determined spectrophotometrically. Leaves were cut into small pieces, mixed thoroughly and 0.25 g of leaves were taken into a mortar to grind them finely by using pestle with 25 ml of 80% acetone for 5 minutes. The homogenate was filtered through filter paper (Whatman No.42) and was made a volume of 25 ml with 80% acetone. The total Carbohydrates were determined by Anthrone method, total proteins by Biuret method and peroxidase activity by O-diansidine method enzymatically (Ganesh *et al*., 2009; Ozdener & Aydin, 2011; Gandhi *et al*., 2017).

**Extract Monitoring by Spectrophotometer**

After the extraction, chlorophyll contents were monitored by UV-Vis spectrophotometer (Hira *et al*., 2013). The optical density/absorbance of each solution were measured at 663 and 645 nm against 80% acetone blank in 1 cm quartz cuvette at room temperature. The Arnon's equation was used to calculate the amount of chlorophyll-a, chlorophyll-b and total chlorophyll (a + b) (Amon, 1949; Peralta *et al*., 2001).

Chl a (mg·g−1) = [(12.7 × A663) − (2.69 × A645)] × ml acetone/mg leaf tissue
Chl b (mg·g−1) = [(22.9 × A645) − (4.68 × A663)] × ml acetone/mg leaf tissue
Total Chl = Chl a + Chl b

## STATISTICAL ANALYSIS

Data was statistically analyzed using one-way ANOVA on Graphpad Prism 6.01 software (Gandhi *et al*., 2018). The results were presented as mean ± S.D. (standard deviation) and data from different treatments and control were compared by Duncan's multiple-range test at $p < 0.05$.

## RESULTS & DISCUSSION

**Visual observation and UV-visible spectroscopy**

In the present study synthesis of calcium oxide, nanoparticles by using leaf extract of Ocimum tenuiflorum characterization, antimicrobial activity and their effect on seedling growth of Cicer arietinum was carried out. The leaf extract of Ocimum tenuiflorum

having greenish brown colour at the time of extraction. When nanoparticle synthesis protocol was carried out the colour change was observed to grey-brown followed by turbidity (Figure-4). The color changed aqueous solution allowed for UV- visible spectra analysis indicating the generation of calcium nanoparticles, due to the reduction of calcium ions into calcium oxide nanoparticles via the active molecules present in the leaf extract of Ocimum tenuiflorum which was explained by Ahmad *et al*., 2003 during extracellular synthesis of silver nanoparticles by Fusarium oxysporum fungal extract. As shown in figure-3, characteristic and well-defined spectral band for calcium oxide nanoparticles was obtained at around λ 332 nm (Figure-3), the same type of results interpreted by Mulvaney, 1996. Control calcium chloride solution neither developed the reddish brown color nor did they display the characteristic band, indicating that abiotic reduction of calcium chloride did not occur under the used conditions. The aqueous solution was allowed for incubation. After 48 hours of incubation at 28 ±2°C the precipitation was settled at the bottom of conical flasks which indicates the formation of NP's and analyzed by SEM, EDAX and DLS. The band gap energy of the phytosynthesized CaO nanoparticles was calculated using Planck's equation as follows.

$E = hC/\lambda$
h = Planck's constant ($4.135 \times 10^{-15}$ eV)
C = Velocity of light ($3 \times 10^{8}$ m/s)
$\lambda = 332 \times 10^{-9}$ nm

Band gap energy of CaO nanoparticle synthesized by using leaf extract of Ocimum tenuiflorum is 3.70 eV.

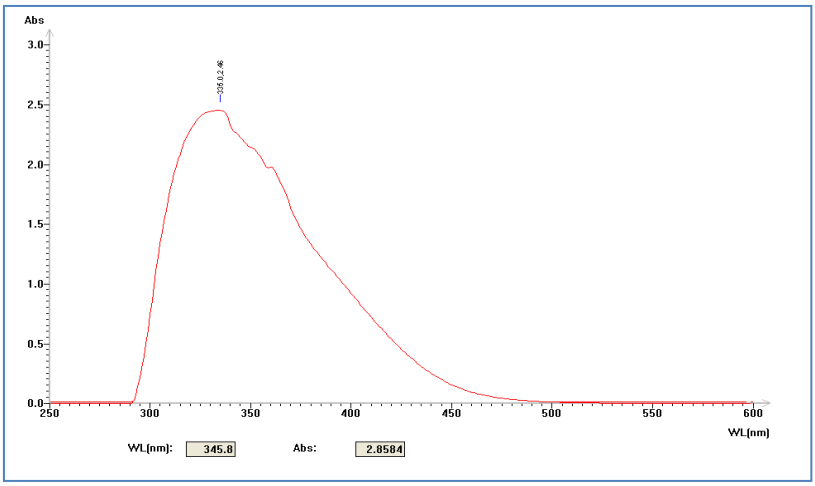


**Fig-3: Uv-visible spectra for calcium nanoparticles synthesized by using leaf extract of Ocimum tenuiflorum after 24 hours incubation at room temperature**

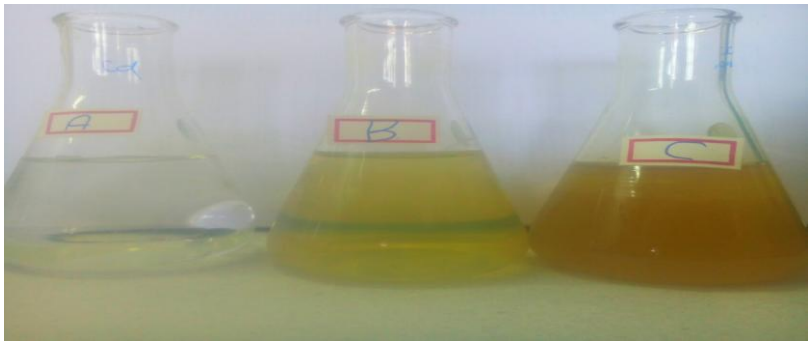


**Fig-4: visual color change observation and formation of calcium nanoparticles using leaf extract of Ocimum tenuiflorum after 24 hours incubation at room temperature A. $CaCl_2$ solution, B. Ocimum tenuiflorum leaf extract and C. mixture of A and B**

**Particle size and distribution**

The phytosynthesized CaO nanoparticles were measured through DLS and characterized by a larger mean size, amounting to 60-70 nm, having a very narrow scatter (standard deviation: 18.4 %). This behavior can be ascribed to the double quantity of leaf extract available for reducing $CaCl_2$, yielding to a more abrupt nucleation and faster growth of the nanoparticles, while producing small agglomerates in some cases. The graphs represented in figure-5, concluding that majority of formed particles were fall into nano range.

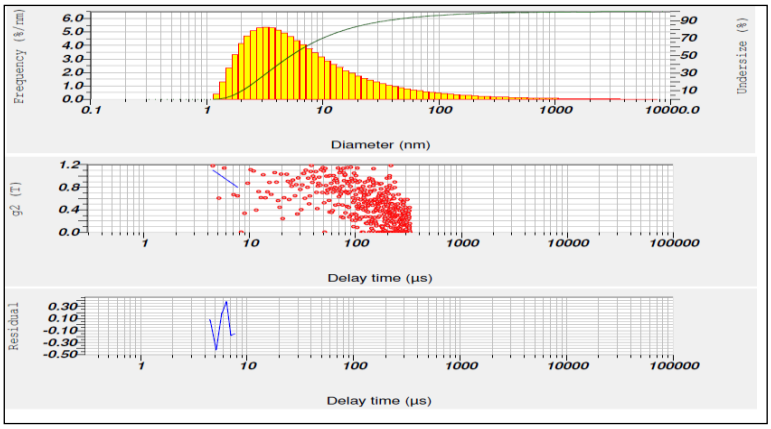


**Fig-5: DLS results for phytosynthesized CaO nanoparticles using leaf extract of Ocimum tenuiflorum.**

**XRD analysis**

Diffraction pattern gives information on translational symmetry - size and shape of the unit cell from peak positions and information on electron density inside the unit cell, namely where the atoms are located from peak intensities. It also gives information on deviations from a perfect particle, if size is less than roughly 100 – 200 nm, extended defects and micro strain from peak shapes & widths.

**Peak Indexing**

Indexing is the process of determining the unit cell dimensions from the peak positions. It is the first step in diffraction pattern analysis. XRD analysis of the prepared sample of CaO nanoparticles was done and data was taken for the 2θ range of 20 to 80 degrees with a step of 0.02 degree. Indexing process of powder diffraction pattern was done and Miller Indices (h k l) to each peak was assigned in first step. Diffractogram of the entire data is in figure-6.

**Calculation of d-Spacing**

The value of d (the inter planar spacing between the atoms) is calculated using Bragg's Law: $2d \sin\theta = n\lambda$

The calculated results were depicted in figure-6.

**Particle Size Calculation**

From this study, considering the peak at degrees, average particle size has been estimated by using Debye-Scherrer formula (Nat *et al*, 2008; Das *et al*, 2009; Nath *et al*, 2007; Hall *et al*, 2000).

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

Where K is instrument constant (0.9), λ is wavelength of X ray diffraction 'β' is FWHM (full width at half maximum), 'θ' is the diffraction angle and 'D' is particle diameter size. The size of nanoparticle was found to be in an range of 30-60 nm from the above calculations (Table-3).

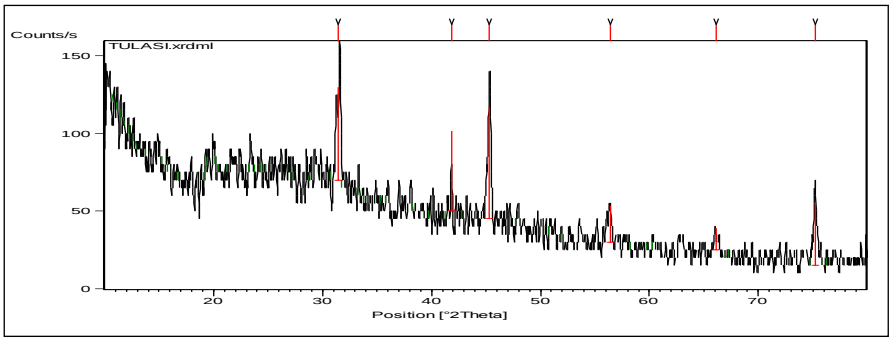


**Fig-6: XRD results for phytosynthesized CaO nanoparticles using leaf extract of Ocimum tenuiflorum**

| Pos. [°2Th.] | Height [cts] | FWHM [°2Th.] | d-spacing [Å] | Rel. Int. [%] |
|---|---|---|---|---|
| 31.4593 | 11.96 | 0.4723 | 2.84375 | 82.96 |
| 41.8224 | 10.29 | 0.2414 | 2.15997 | 71.41 |
| 45.2727 | 14.41 | 0.4723 | 2.00305 | 100.00 |
| 56.4191 | 4.70 | 0.4723 | 1.63093 | 32.62 |
| 66.1700 | 2.80 | 0.2709 | 1.41228 | 19.43 |
| 75.2072 | 8.08 | 0.5760 | 1.26238 | 56.05 |

**Table-1: Water quality parameters (water used for irrigation)**

| S. No | parameter | Analyzed value |
|---|---|---|
| 01 | pH | 6.9 to 7.2 |
| 02 | EC | 130.00 µmhos/cm |
| 03 | Salinity | 0.03 ppt |
| O4 | Dissolved oxygen | 9.72 mg/L |
| 05 | Nitrate Nitrogen | 0.84 mg/L |
| 06 | Nitrite Nitrogen | 0.02 mg/L |
| 07 | Total hardness | 84 mg/L |
| 08 | Sulfates | 23 mg/L |
| 09 | Chlorides | 2.7 mg/L |
| 10 | Calcium | 17 mg/L |
| 11 | Magnesium | 8 mg/L |

**Table-2: Soil quality parameters (soil used for seed germination and growth analysis)**

| S. No | parameter | Analyzed value |
|---|---|---|
| 01 | pH | 7.2 |
| 02 | EC | 0.78 µmhos/cm |
| 03 | Moisture (%) | 3.2 |
| O4 | CEC | 172 meq/100 g |
| 05 | Nitrate Nitrogen | 0.19 mg/ 100 g |
| 06 | Nitrite Nitrogen | 0.12 mg/100 g |
| 07 | phosphorus | 0.53 mg/100 g |
| 08 | Sulfates | 4.3 mg/ 100 g |
| 09 | potassium | 77 mg/ 100 g |
| 10 | Calcium | 1.2 mg/ 100 g |
| 11 | Magnesium | 0.7 mg/ 100 g |

**Table-3: Particle size calculations**

| Peak position (2θ) | Particle Size (nm) |
|---|---|
| 31.45 | 29.4 |
| 41.82 | 12.2 |
| 45.27 | 36.6 |
| 56.41 | 29.4 |
| 66.17 | 54.98 |
| 75.20 | 24.1 |

**Scanning Electron Microscope analysis**

Figure-7 shows the SEM image of CaO nanoparticles. The bright areas of the picture reveal high emission of secondary electrons when exposed to electron beam of SEM. This is due to high surface to volume ratio in those areas (Roy, 2010). The SEM morphology revealed the agglomeration of CaO nanoparticles. It can be seen from the micrograph that the synthesized sample composed of grains with no regular shape. The average grain size of CaO nanoparticles varies from few nm to 0.5 microns. The interaction between nanoparticles with large surface area and high surface energy results in agglomeration (Blanton & Barnes, 2005). Individual particles seem to be nano sized plate/rod like crystals and they partially fused to form hard agglomerates. The components of the products were further confirmed by EDX, which indicates that the ratio of Ca, Si and Cl shown in figure -8 (a) & 8 (b) and values (%) depicted in table – 4 & 5.

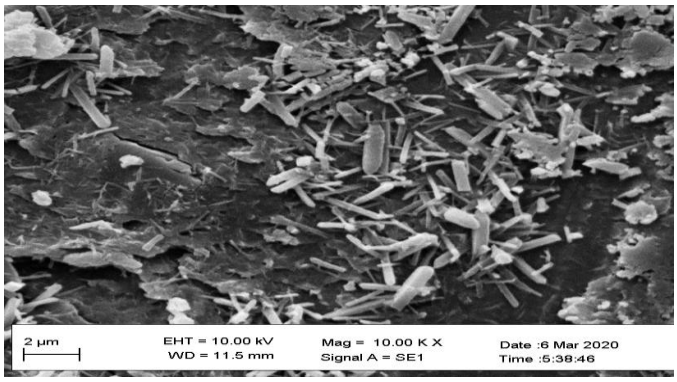


**Fig-7: SEM images of phytosynthesized CaO nanoparticles using leaf extract of Ocimum tenuiflorum**

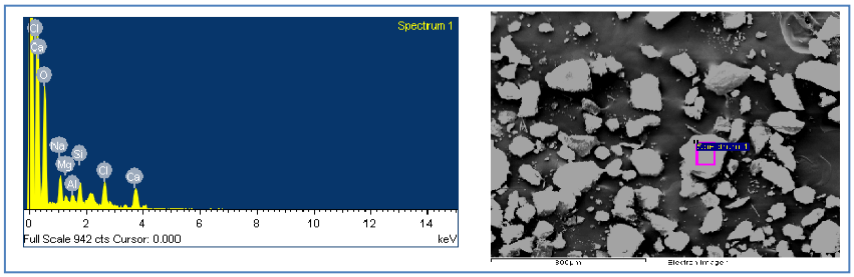


**Fig-8 (a): EDAX image of synthesized calcium oxide nanoparticles**

**Table-4: Elemental Analysis of EDAX**

| S.No | Element | Weight (%) | Atomic (%) |
|---|---|---|---|
| 01 | O K | 60.50 | 75.48 |
| 02 | Na K | 6.10 | 5.29 |
| 03 | Mg K | 1.32 | 1.08 |
| 04 | Al K | 1.23 | 0.91 |
| 05 | Si K | 5.67 | 4.03 |
| 06 | Cl K | 10.30 | 5.80 |
| 07 | Ca K | 14.88 | 7.41 |
| 08 | **Total** | **100** | **100** |

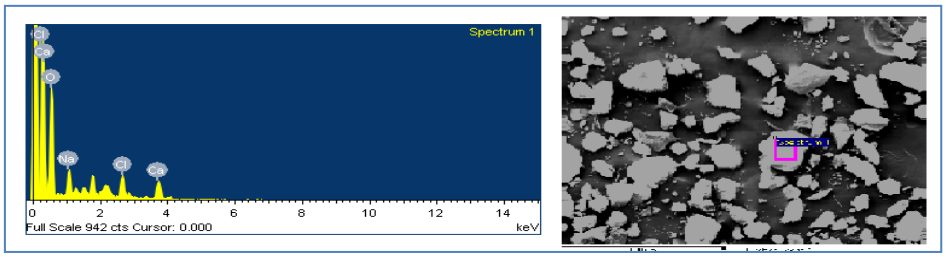


**Fig-8(b): EDAX image of synthesized calcium oxide nanoparticles**

**Table-5: Elemental Analysis of EDAX**

| S.No | Element | Weight (%) | Atomic (%) |
|---|---|---|---|
| 01 | O K | 65.48 | 79.90 |
| 02 | Na K | 7.13 | 6.06 |
| 06 | Cl K | 11.13 | 6.13 |
| 07 | Ca K | 16.25 | 7.92 |
| 08 | **Total** | **100** | **100** |

**Anti- microbial activity**

Prepared CaO nanoparticles were tested for its antimicrobial activity with gram +ve (Bacillus subtilis, Staphylococcus aureus) and gram -ve bacteria (Pseudomonas aeruginosa, Escherichia coli). Prepared sample was found to be antimicrobials (Table-6). The nanoparticles of CaO show good antibacterial properties because of large surface area and the reduced particle size increases the reactivity of CaO nanoparticles with gram positive and gram negative bacteria used in current investigation. Due to smaller size, these nanoparticles can easily enter in the bacterial cells and an inhibition mechanism proceeds inside the bacterial cell. After entering in the bacterial cell, a CaO nanoparticle leads the distortion and destroys the cell membrane that causes the death of cells (Ramola *et al*., 2019). The solution which used for nanoparticles synthesis doesn't shown any zone of inhibition. The CaO nanoparticles only shown zone of inhibition against both gram positive and gram negative bacteria along with streptomycin which was used as the positive control. The MIC of CaO nanoparticles were tested andfound that 5µg/mL shown inhibition which indicated phytosynthesized CaO nanoparticles can shows MIC at very minute concentrations.

**Table-6: Anti-microbial activity of phytosynthesized CaONP**

| S.No | Organism | Zone of inhibition (mM) |
|---|---|---|
| 01 | Escherichia coli | 13.6 |
| 02 | Pseudomonas aeruginosa | 14.2 |
| 03 | Bacillus subtilis | 12 |
| 04 | Staphylococcus aureus | 9 |

**Agricultural Application of CaO nanoparticles**

All the treatments led to 100% germination of seeds showing that nano-CaO particles did not adversely affect the seed germination. Seed germination is the beginning of a physiological process that needs water imbibitions (Gandhi *et al*., 2020). It was also noted from the experimental results that CaO nano particles promoted the growth of roots hairs; these being important for the uptake of immobile nutrients such as Phosphorus. The seedling growth rate were calculated every 15 days for a period of 30 days, results were calculated and shown in figure-9 (shoot growth) figure-10 (root growth). From the figures it is observed that CaO nanoparticles enchanced the growth rate of seedlings. CaO 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 which resulting increase in fresh bio mass of seedling (figure-11) and dry weight (figure-12). 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 plant stress parameters i.e. % phtotoxicity (figure-13), % inhibition (figure-14) and tolerance indices (figure-15), seedling vigor index (figure-16) were calculated according to formula which described above. From the obtained results it was clear CaO nanoparticles which used in present study doesn't shown any negative impact or toxicity with respect to growth parameters. As concentration of CaO increased the seedling growth increased and toxicity levels were decreased. The similar types of results were obtained by Yugandhar & Savithramma 2013, with respect to $CaCO_3$ nanoparticles impact on seed germination and seedling growth response on Vigna mungo (L.). The results also concluding that utilization of CaO nanoparticles prepared by leaf extract of Ocimum tenuiflorum will give an economical solution to farmers for enhance the growth rate of Bengal gram (Cicer arietinum).

The seedling growth analysis parameters such as Relative Growth Rate (RGR), Net Assimilation Rate (NAR), Leaf Area Ratio (LAR), Leaf Weight Ratio (LWR), Specific Leaf Area (SLA), Specific Leaf Weight (SLW), Leaf Area Duration (LAD) of Bengal gram (Cicer arietinum L.) seedlings were estimated and the CaO nanoparticles showed increased growth with increase of concentration and crop growth periods, compared to control treatment. The similar type of reports interpreted by Gandhi *et al*., 2020 carried experiments with stress of edaphic factors.

All the biochemical parameters i.e. total carbohydrates, total proteins, photosynthetic pigments and POD enzyme activities were determined with respect to all treatments and results were shown in table-7. From the results it is observed that all the biochemical content increased with increase in concentration of CaO nanoparticles (Gandhi *et al*., 2019). Several studies are concerned with the synthesis of nanoparticles using biological routes and promotory effects on seedling growth. So far very few studies have been reported on the biological synthesis and promontory effects of calcium oxide nanoparticles on plants. Hence the present study was undertaken to synthesize CaO nanoparticles and know the effect on seed germination and seedling growth of Bengal gram (Cicer arietinum L.). The results concluded that CaO nanoparticles can be use as inorganic fertilizer in the fields of Bengal gram (Cicer arietinum L.).

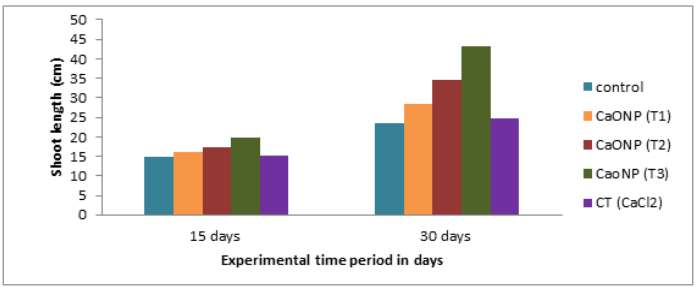


**Fig-9: Impact of CaONP concentrations on shoot growth of Bengal gram (Cicer arietinum L.).**

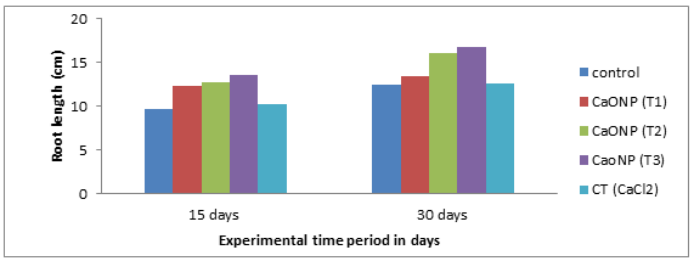


**Fig-10: Impact of CaONP concentrations on root growth of Bengal gram (Cicer arietinum L.).**

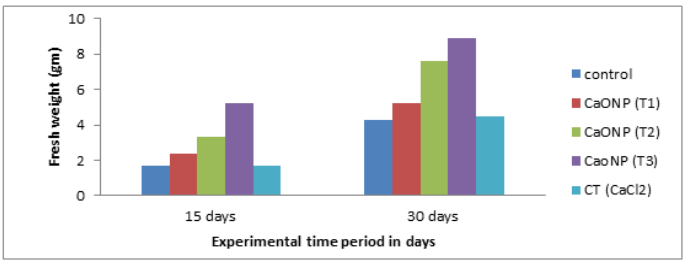


**Fig-11: Impact of CaONP concentrations on fresh weight of Bengal gram (Cicer arietinum L.) seedlings**

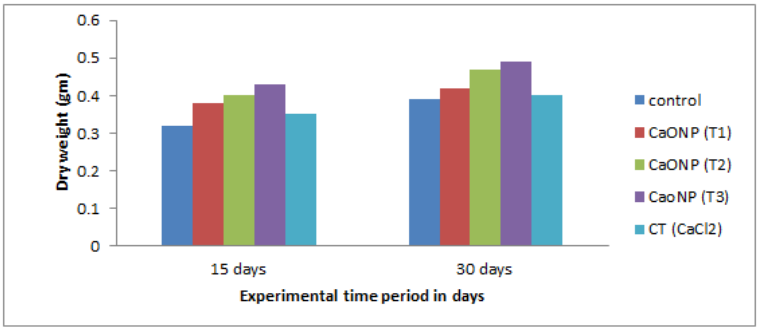


**Fig-12: Impact of CaONP concentrations on dry weight of Bengal gram (Cicer arietinum L.) seedlings**

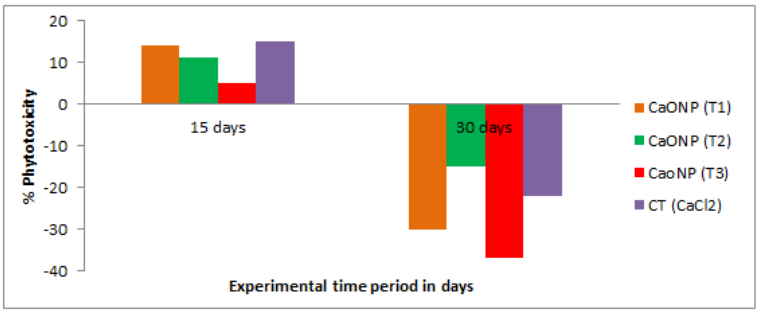


**Fig-13: %phytotoxicity of synthesized CaONP on seedlings of Bengal gram (Cicer arietinum L.)**

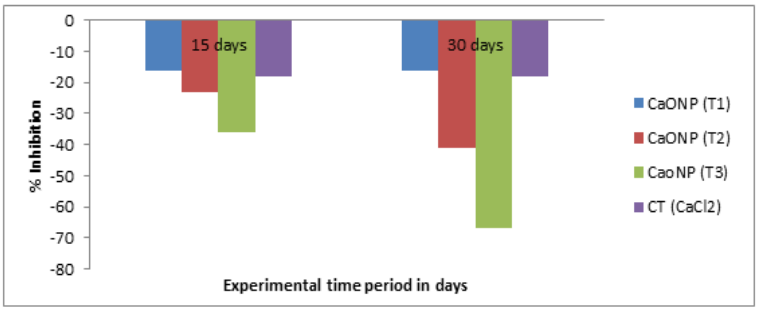


**Fig-14: % inhibition on seedlings growth of Bengal gram (Cicer arietinum L.) at various concentrations of synthesized CaONP**

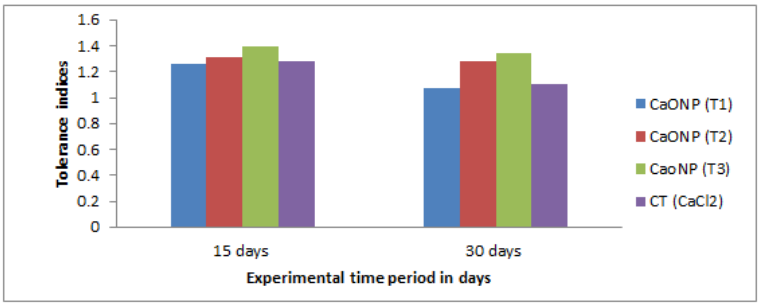


**Fig-15: Impact of CaONP on tolerance indices of seedling growth of Bengal gram (Cicer arietinum L.)**

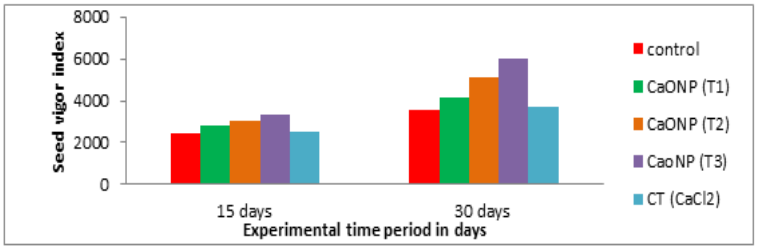


**Fig-16: Impact of CaONP on seed vigor index of Bengal gram (Cicer arietinum L.).**

**Table-7: Impact of synthesized CaONP on biochemical properties & chlorophyll content (a, b and total) of Bengal gram (Cicer arietinum L.) seedlings.**

| **S.No** | **Total Carbohydrates (mg/g)** | **Total proteins (mg/g)** | **POD enzyme activity (mg/g)** |
|---|---|---|---|
| Control | 36.98 | 8.26 | 11.59 |
| T1 | 47.91 | 8.92 | 15.45 |
| T2 | 48.52 | 9.49 | 20.54 |
| T3 | 52.84 | 11.62 | 33.75 |
| CT | 42.84 | 8.99 | 12.62 |
| **S.No** | **Chlorophyll-a** | **Chlorophyll-b** | **Total chlorophyll (a+b)** |
| Control | 6.98 | 0.99 | 7.97 |
| T1 | 7.67 | 1.39 | 9.06 |
| T2 | 7.99 | 1.42 | 9.41 |
| T3 | 8.35 | 1.76 | 10.11 |
| CT | 7.55 | 1.15 | 8.70 |

## CONCLUSIONS

The present study concluded that the CaONP's are synthesized in biological friendly pathway and they have positive growth promontory effects on growth of Bengal gram (Cicer arietinum L.) seedlings. The synthesized nanoparticles under lab conditions are observed that they play an important role in seed germination and seedling growth of Bengal gram (Cicer arietinum L.). 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 at open field level. Because most of the micro organisms are sensitive to nanoparticles. Results of present study showed that, this green synthesis method can be applied for rapid, cost effective, and eco-friendly way for the synthesis of calcium oxide nanoparticles which can be used further in various industrial and medical applications along with agricultural development.

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