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Microwave Mediated Green Synthesis of Silica Nanoparticles, Characterization, Antimicrobial Activity, Promising Applications in Agriculture

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Abstract—The current study describes a green method for synthesis of silica nanoparticles (SiNPs) from banana peels (*Musa paradisiaca*). Utilizing the Ultraviolet-Visible (UV-Vis) Spectrophotometer, Fourier Transform Infrared (FT-IR), Dynamic Light Scattering (DLS) and Zeta, X-ray Diffractometer (XRD), Scanning Electron Microscope (SEM), and Energy Dispersion Spectroscopy (EDS), these SiNPs were characterized and their effectiveness at inhibiting the growth of various microorganisms was when these SiNPs were synthesised, they displayed a colour change pattern, and a UV-Visible spectrophotometer analysis revealed a broad peak at 365 nm. The presence of Si content, as well as the appearance of phytochemicals such primary amines of proteins and significant amounts of fibre, were revealed by FT-IR analysis to be the essential factors in the capping and stabilisation of SiNPs. Nanoparticles had an average size of 45 nm and a zeta potential value of 21.3 mV, according to DLS and zeta potential measurements. An XRD analysis revealed a broad peak at 22^{o} and 26^{o} of 2ɵ value, confirming the amorphous nature of the nanoparticles and their average size range of 35 nm. The particles were poly-dispersed, spherical in shape, and ranged in size from 7 to 60 nm with negligible agglomeration among the particles, according to higher magnification examinations with SEM analysis. A 45.22 weight percentage of silica was found in the sample by energy dispersive X-ray analysis, which points to the sample's extreme purity. Gram positive and gram negative bacteria are used to test the produced nanoparticles for growth inhibitory action on various microorganisms, potentially showing inhibitory activity. The fruit peel of *Musa paradisiaca* was shown to be an effective and dependable green source for the manufacture of possible bio antibacterial SiNPs, according to the study's findings. The current study also offers an accurate explanation of the physiochemical and biological approach of silica nanoparticles in plants, which promotes safer and more environmentally friendly agriculture and better plant growth.

Keywords— *Musa paradisiaca* fruit peel (Banana), silica nanoparticles, microwave, FTIR, XRD, SEM, EDX, Uv-visible spectrophotometer, antimicrobial activity and physiological, biochemical analysis.

I. INTRODUCTION

Nanoparticles are significant in technology, medicine, agriculture, and the reduction of environmental pollution because of their distinctive catalytic, electronic, magnetic, chemical, photo-electrochemical and optical capabilities [1, 2]. They often have varied forms, no distinct toxicities, and special optical and electrical capabilities, making them useful in optics, electronics, catalysis, and biomedicine. In addition to this, mineral and metal ion nanoparticles are utilized as nano-fertilizers to boost crop output while lowering cultivation costs [3]. With limited land and water resources, boosting resource usage efficiency while causing the least amount of harm to the soil and ecosystem is the only option to enhance crop yield. Nanotechnology, a new and developing field of research, has the potential to replace the traditional agricultural system and so offer a solution to the most pressing issues facing modern agriculture [4].

II. RELATED WORK

The innovative idea for enhancing the quality of the agricultural system is the use of nano-fertilizers, which have numerous advantages over conventional fertilizers. Although silicon is one of the four useful elements, it is not regarded as an essential nutrient for the growth and development of plants [5]. Under biotic and abiotic stressors, it is critical for plants. In light of this, the current work covers the silica nanoparticles green synthesis, characterization, antimicrobial activity, and application in agriculture.

III. METHODOLOGY

General description about plant material

Musa paradisiaca (Figure-1) is a sterile triploid. Cultivated in warm climates for its tasty yellow peeled fruit called as banana. Banana fruits are rich in dietary fibers & carbohydrates, which helps in various body metabolisms of living organisms. The peel of banana is a part of fruit that generally is discarded considering as waste in many parts of the world. In present investigation banana peel used for preparation of silica nanoparticles to increase the utilization of discarded waste from bananas. which also helps in controlling environmental pollution and improves the solid waste management practice. The 100 gm of banana peel powder consist of 0.48 % silica, 31.7 % crude fiber and 72.71 % cellulose [6]. Thus taking the nutritional content of banana peel into consideration banana peel has been selected as source, for synthesis of silica nanoparticles to use as nano-fertilizer for improvement of crop growth.

Collection of plant material

Arati is the regional name for *Musa paradisiaca*, an annual fruiting angiosperm that is a member of the musaceae family. The plant *Musa paradisiaca* has significant nutritional qualities and is rich in silica content, according to Indian mythology and nutritional scenarios. The *Musa paradisiaca* fruits are purchased from the neighbourhood market in the research location and allowed to mature to the point of being ready for harvest. After being cut off from the fruit, the peel is dried for 10 days in the shade. The dried banana peels were broken up into tiny bits, crushed into a fine powder, then sieved using an Indian-grade 250 mic mesh sieve. The powder is then kept in a plastic container at room temperature until further use.

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Preparation of SiNPs

After the synthesis of SiNPs, the Adam et al., [5] methodology is slightly modified. 50 g of finely ground *Musa paradisiaca* fruit peel were combined with 500 ml of 1 M HNO_3 in a 1000 ml Erlenmeyer conical flask, and the mixture was agitated for 24 hours at a standard 200 RPM using a magnetic stirrer. To raise the pH of this mixture to 4.0–5.0, it is filtered and then rinsed with distilled water. For 12 hours, this solution was dried in an oven set between 100 and 110^{o}C. To achieve a pH of 12, the evaporated turbid solution was mixed with 500 ml of 1 M NaOH solution for 24 hours using a magnetic stirrer. The resulting solution was then microwaved at 700 watts for six hours. The resulting reaction mixture is separated using a suction pump, and the pH is then titrated with 3 M HNO_3 until it reaches 8.5 to 9.0. To separate biological admixtures, the contents were centrifuged at 5000 RPM for 10 min. After that, they were cleaned three or four times with distilled water and dried at 80^{o}C in a hot air oven for 12 h. The resulting powder was thoroughly mashed with a mortar and pestle and then subjected to antibacterial and characterization tests.

Characterization of SiNPs

Before drying, the produced nanoparticles underwent ultraviolet-visible (UV-Vis) spectroscopic analysis using a UV-Vis Spectrophotometer Lambda, India, between the scan ranges of 200 and 700 nm. An ALPHA interferometer (ECO-ATR), ZnSe liquid transmission cell, and KBr pellet technique were used to study Fourier-Transform Infrared (FT-IR) spectra in the scan range of 4000 to 500 cm^{-1}. The Japan-made Horiba SZ 100 nanoparticles analyzer was used to conduct dynamic light scattering (DLS) and Zeta potential experiments. With a Siefert computerized X-ray diffractometer 3003 TT, an X-ray diffraction (XRD) examination was performed using CuK1 radiation at 40 kV and 20 mA at the 2ɵ range between 10° and 80°. Nanoparticles elemental analysis and scanning electron microscopic (SEM) examination were carried out on Hitachi S-4500 SEM attached with EDAX machine (Hitachi, Mexico) operating at 15 kV passage of electric voltage.

Phytosynthesis of Anti-microbial Si Nano particles

After microbial cultures were collected from the School of Life Sciences, Jawaharlal Nehru Institute of Advanced Studies, Hyderabad, they were used to analyze the antimicrobial activity of prepared Si nanoparticles. These major groups of microorganisms included gram-positive bacteria and gram-negative bacteria. Bacterial strains were incubated at 4^{o}C on nutrient agar slants. Testing was done to see how effective antimicrobial Si nanoparticles were. Finding the Minimum Inhibitory Concentration (MIC) of the Si produced nanoparticles is the first test. The second test uses the well-diffused agar method to measure the antibacterial activity of the three solutions SiO_2 aqueous solution, peel powder extract, and final processed Si nanoparticles against gram positive and gram negative bacteria.

Minimum Inhibitory Concentration

The lowest minimum inhibitory concentration of Si nanoparticles, which prevents test organisms from growing visibly, was determined. Test organism suspensions were permitted to be swabbed on the culture media. Si nanoparticles (5, 10, 15, and 20 g/mL) were introduced in various concentrations to the wells that were plated with sculptures. The plates were incubated at 37^{o}C for roughly 24 hours to detect the inhibiting growth of Si nanoparticles. The subsequent process was carried out three times, and the mean value was then noted.

Well diffusion assay

The well diffusion agar plate method was used to determine the activity of the phytosynthesized Si nanoparticles. With the use of freshly made bacterial cultures of gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria from 15 sterilized plates, about 12 plates were infected. One plate is kept under negative (-ve) control while the other two plates were kept under control (+ve). Using a stainless steel cork borer, four wells of agar measuring 6 mm in diameter were strained in all of

the plates. Each well has a distinctive Group A, B, C, and D designation. *Musa paradisiaca* leaf and fruit peel extract, each at a concentration of 20 µg/l, were added to wells A and B, respectively. The filtered, final concentration of phytosynthesized Si nanoparticles (20 µg/ml) was added to well C. As a positive control, well D was filled with 20 µg/ml of streptomycin. At a temperature of 37°C, the plates were incubated for 24 hours. After that, the area around the wells that was inhibited was noted. The values were assigned using Microsoft Office Excel 2007, and statistical analysis was performed on them using GraphPad Prism version 6.0. The data were compared both within and across groups using a one-way ANOVA test with a 5% threshold of significance.

Promising Application of Si Phytosynthesized Nanoparticles in Agriculture

The tests was place in Green Fields Institute of Agriculture Research & Training, Mangalpalli Ibrahimpatnam, Rangareddy, Telangana, India for roughly 30 days between January and February, or (05/01/2020 to 05/02/2020). Soil samples were taken from open fields at the research facility during the month of December 2019 in order to evaluate the effects of Si phytosynthesized nanoparticles on Bengal gram (*Cicer arietinum*). The gathered soil samples were clear of weeds and other solid undesirable things, and they were allowed to dry in the sun for roughly four days. According to the standardized testing procedures outlined in the APHA 2nd edition, the soil samples were allowed for physical and chemical examination. The remaining dirt was then transferred into polypropylene tubs for additional demonstrations [7, 8]. Results of the physical-chemical examination of the composite soil sample that was collected are shown in table 6.

Equal amounts of dirt, or 2 kg each tub, were placed in a series of 12 polyethylene containers, each of which was labelled NC (Negative or Control), T1 (lower concentration), T2 (medium concentration), and T3 (greater concentration), as well as PC (Presoaking of seeds in 10 mM SiO_2 solution termed as positive control). The mean data from a series of experiments were taken for analysis.

Selection of Seed & Seed Treatment

In the current study, Bengal gram (*Cicer arietinum*), also known as chickpea, seeds were employed. The seeds were purchased from a local market in Ibrahimpatnam and were certified and processed.

30 seeds were presoaked in solution-A containing 50 ml of full strength phytosynthesized Si nanoparticles (well sheared with double distilled water) and the amount was increased to 250 ml using distilled water as T1. A second batch of 30 seeds were soaked in solution B, which is made by combining 250 ml of double-distilled water with 100 ml of Si nanoparticles solution. This was deemed to be T2. Third batch of 30 seeds were placed in solution C, which was created by mixing 150 ml of silica nanoparticles with 250 ml of double-distilled water to create T3. A group of 30 seedlings were let to sprout in regular soil as a negative control (NC). A second batch of 30 seeds, known as the positive control treatment (PT), or silicon treatment, was presoaked in 250 ml of a 10 mM SiO_2 solution for 12 hours. Pre-soaked T1, T2, T3, and PT Bengal gram (*Cicer arietinum*) seeds were distributed randomly into the appropriate tubs and watered with groundwater right away. Irrigation water was made available for physicochemical examination [9–11], and the results are shown in table 7. Every experimental tub received a daily irrigation to keep the soil moist to the point of saturation starting at the time of seeding. For greater light and air, the trials were set up in open space.

Growth evaluation

To calculate various growth parameters and represent the instantaneous values and mean values over a period of time, the following information is needed. The terms W, WL, WS, and WR are used to denote the dry weights of the plant as a whole, the dry leaves, the stem, and the roots, respectively. A is the leaf area in contrast.

Relative Growth Rate (RGR)

Blackman is the one who first used the phrase RGR. It is described as the rate at which dry matter is added for every existing unit of dry matter. Since the rate of growth is expressed as the rate of interest on the capital, this is also known as the "Efficiency Index." It offers a useful general indicator of plant growth. The following formulas can be used to determine RGR [12].

$$Relative\ Growth\ Rate = \frac{loge^{W_2} - loge^{W_1}}{T_2 - T_1}$$

Net Assimilation Rate (NAR)

The NAR is an estimation of the net photosynthetic carbon ingested by photosynthesis minus the carbon lost by respiration. It measures the quantity of photosynthetic product entering plant material. The NAR is often expressed as grams of dry weight gain per square centimetre of leaf surface during a specific time period and can be calculated by periodically measuring plant dry weight and leaf area throughout growth. Because only the active leaf area is included in the assimilatory area when calculating the rate of dry matter generation, this is also known as unit leaf rate. Given a time range from T1 to T2, the mean NAR is given by

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

Leaf Area Ratio (LAR)

The LAR is a measurement of the percentage of a plant that is actively involved in photosynthetic activity. It indicates the assimilatory apparatus's relative size. It is additionally known as the capacity factor. It is described as the proportion of leaf area (measured in square centimetres) to the total dry weight of the plant. On an area basis, it represents the leafiness characteristic of crop plants [13].

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

Leaf Weight Ratio (LWR)

The ratio of the grams of dry matter in leaves to the total amount of dry matter in plants is one of the elements of LAR. LWR is dimensionless since the numerator and denominator are calculated using dry weight. It measures the plant's leafiness on a weighted basis.

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

Specific Leaf Area (SLA)

It is a part of LAR and is calculated as the ratio of the total leaf dry weight in grams to the leaf area in cm^2. This is a metric for determining leaf density. These steps can be used to compute the mean SLA

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

Specific Leaf Weight (SLW)

SLA's inverse is known as SLW. It is described as the relationship between leaf area and total dry weight. It displays the relative leaf thickness of various genotypes.

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

Leaf Area Duration (LAD)

It is a measurement of the total leaf area over time. The size of the leaf area and the timing of its application are taken into account. It also symbolizes how leafy the crop was during the growing season. As a result, the leaf area duration measurement can be made over the course of a day, a week, or even a month [14].

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

Plant Sampling and Analysis

When a root extends more than 2 mm, a seed is deemed to have germinated. The term "seed germination rate" refers to the average number of seeds that sprout over periods of five or 10 days. The following formula [15] can be used to calculate tolerance indices and germination percentage.

$$\%\ of Germination = \frac{Number of Seeds Germinated}{Total Number of Seeds Planted} X\ 100$$

$$Germination\ Index\ (GI) = \sum_{i=1}^{k} \frac{No.of\ germinated\ seeds}{the\ count\ day}$$

Where i=1 day one, k is the last day of observation.

$$Mean\ Germination\ Time\ (MGT) = \frac{\sum_{i=1}^{k} n_i\ t_i}{\sum_{i=1}^{k} n_i}$$

Where t_i is the time from day one to the last day of observation, n_i is an observed number of germinated seeds every day and k is the last germination day of observation.

$$Mean\ Germination\ Rate\ (MGR) = \frac{1}{Mean\ Germination\ Time}$$

$$Co-efficient\ of\ variation\ of\ the\ time = \frac{S_t}{Mean\ Germination\ Time} X\ 100$$

Where S_t is standard deviation of germination time

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

The suppression of seedling growth was expressed according to the formula [16, 17].

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

Seedling Vigor Index

The characteristics of a seed that define its level of activity and performance during germination and seedling emergence are known as the seedling vigour index. It is a single quantifiable feature, similar to germination that describes a number of traits linked to various elements of seed performance. The following formula is used to determine seedling vigour index [18, 19]

SVI = Germination percentage × Seedling length

Percentage Phyto-toxicity

Calculations of the percentage phytotoxicity of heavy metals on the root and shoot development of Bengal gram (*Cicer arietinum*) were made at regular intervals (5 to 30 days of seedling growth). The percentage of phytotoxicity was calculated using the formula shown below [20, 21].

$$Percentage of Phytotoxicity = \frac{\frac{S}{R}lengthofcontrol - \frac{S}{R}lengthoftreatedseed}{\frac{S}{R}lengthofcontrol} X\ 100$$

Estimation of Biochemical Attributes

Photosynthetic pigments were explored in terms of biochemical characteristics. Spectrophotometric analysis was used to calculate the amounts of chlorophyll-a, chlorophyll-b, and total chlorophyll (a + b). The leaves were divided into small pieces, well mixed, and 0.25 g of leaves were added to a mortar and pestle along with 25 ml of 80% acetone to crush the leaves into a fine powder for 5 minutes. The homogenate was made up to a volume of 25 ml with 80% acetone and filtered using filter paper (Whatman No. 42). Total protein concentration was assessed using the Biuret method, total carbohydrates by the Anthrone method, and peroxidase activity by the O-diansidine method enzymatically [22–24].

Extract Monitoring by Spectrophotometer

After the extraction, chlorophyll contents were monitored by UV-Vis spectrophotometer [25]. 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) [26]:

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
One-way ANOVA was used to statistically evaluate the data on Graphpad Prism 6.01 [13,14]. Results were provided as mean S.D. (standard deviation), and Duncan's multiple-range test was used to compare data from various treatments and the control group at a p-value of 0.05.

IV. RESULTS AND DISCUSSION

Ultraviolet–visible (UV–Vis) spectroscopic analysis
When the final SiNP solution combination was analysed using ultraviolet-visible (UV-vis) spectroscopy in the spectra that were recorded, it revealed an observable peak at 365 nm, which is the wavelength of the surface plasmon resonance of SiNP (Figure-2). The resonance peak of silica nanoparticles is observed in this area, according to numerous reports [27]. The band gap energy of the Si nanoparticles produced through phytosynthesis was determined using Planck's equation. The formula for the equation is

$E = hC/\lambda$
h = Planck's constant (4.135×10^{-15} eV)
C = Velocity of light (3×10^{8} m/s)
$\lambda = 360 \times 10^{-9}$ nm

Band gap energy of Si nanoparticle is synthesized by using the extract of *Musa paradisiaca* fruit peel is 9.57 eV.

Fourier Transform Infrared (FT-IR) Analysis
The dried banana peel powder's FTIR spectra (Figure-3) show that there are several functional groups in its biomass, showing the complexity of the raw material used to create the silicon nanoparticles. The presence of various functional groups was shown by the transmittance peak at various frequencies. High peaks caused by -OH stretching were seen at frequencies of 3652, 3412, and 3268 cm^{-1}. These strong peaks indicated the presence of free hydroxyl groups in polymeric materials like lignin or pectin that were deceiving the functional groups of alcohols, phenols, and carboxylic acids. Free hydroxyl groups are allocated a wide frequency range between 3600 and 2800 cm^{-1}, indicating the presence of polymeric molecules (21, 22). At 1618 cm^{-1}, primary amines' N-H bending vibrational peaks were visible. The C=O stretching vibrations of carboxylic groups (-COOH, $-COOH_3$), which can be attributed to carboxylic acids or their esters, have a peak at 1990 cm^{-1}. When nanoparticles are synthesized, there is a difference in transmittance at these peaks (Figure 4), which suggests that the caboxylic group is engaged in particle reduction and capping mechanisms. The peak at 1413 cm^{-1} might be caused by the lignin's aromatic ring vibrating. The peak at wave number 1147 cm^{-1} is attributed to stretching vibrations of the C-N bond of aliphatic amines, while the peaks at wave numbers 1242 cm^{-1} and 1364 cm^{-1} are attributed to C-H bending of crystaline cellulose and C-H bending of cellulose, hemicellulose, or lignin polymer, respectively [28].

The silica nanoparticles produced by phytosynthesis have a straightforward and assigned FTIR spectrum (Figure 4). The Si-O-Si stretch of silica is thought to be responsible for the significant transmittance peak at 1105 cm^{-1}. Si nanoparticle production is thereby controlled. The surface hydroxyl groups of phytosynthesized silica are responsible for the transmittance maxima at 1630 cm^{-1} and 3327 cm^{-1}. The FTIR spectra of dried banana peel powder revealed that it contains hydroxyl, carboxylic, and amine functional groups. The decrease in transmittance and slight deflection in the band frequencies following the synthesis of nanoparticles at these peaks demonstrate the involvement of these functional groups in the synthesis of nanoparticles [29, 30].

Particle Size Distribution (DLS)
Si nanoparticles that were produced through phytosynthesis were measured using DLS and were distinguished by a greater mean size that reached 70 nm and a very narrow scatter (standard deviation: 18.4%). These silica nanoparticles are 35 nm in size, according to the dynamic light scattering (DLS) size distribution histogram. Some distributions at the smaller end of the particle size spectrum show that the synthesized particles are ejecting into the smaller end of the particle size spectrum. Some distribution of larger particle sizes suggests that manufactured particles also exhibit larger particle sizes in some manner. Similar kinds of outcomes were seen in Gandhi et al., 2018 study [31] for the synthesis of lead (Pb) nanoparticles utilizing an extract from *Cuminum cyminum* seed powder. The DLS pattern of a dispersion of silicon nanoparticles made from *Musa paradisiaca* fruit peel is shown in Figure-5. Zeta potential investigation finds a negative value of 21.3 mV.

X-ray diffraction and energy dispersive X-ray spectroscopy analysis
X-ray diffraction pattern (XRD) was recorded for the synthesized Si NPs (Figure-6). Various distinct diffraction peaks at 15.93^{o}, 22.08^{o}, 26.65^{o}, 30.09^{o}, 34.29^{o}, 35.41^{o} and 59.81^{o} were observed. Similar results were reported for Si NPs in the literatures of Haribabu et al., 2018 [27].

Peak Indexing
It is the process of determining the unit of cell dimensions, from the peak positions. It is the first step in diffraction pattern analysis. In XRD analysis, a prepared sample of Silica Nanoparticles was done and data was recorded for the 2θ range of 20 to 80 degrees with a step of 0.02 degree. In Indexing process, powder diffraction pattern was done and Miller Indices (h k l) to each peak was assigned in first step. Entire data of diffractogram is depicted in the Figure-6.

Calculating of d-Spacing
By using *Bragg's Law*, the value of d is calculated as $2d\sin\theta = n\lambda$ [32]

The results are depicted in table-1.

Calculating Particle size

By using *Debye-Scherrer formula* the average particle size has been estimated by considering the degrees at peak. [33-36]

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

Where K is constant (0.9), λ is the 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 a range of 40-70 nm from the above calculations. Similar types of results were observed with Gandhi et al., 2018 [32] for the preparation of copper nanoparticles using *Piper nigrum* seed extract.

SEM and EDX Analysis

Polydispersed, semi-spherical particles with a size range of 50 to 70 nm were discovered by 200 nm resolution investigations of SEM analysis, and the majority of the particles had settled in an agglomerated state (Figure-7). An energy dispersive X-ray study revealed 54.25% oxygen and a weight percentage of silica of 43.62. (Figure-8). The sample's silica concentration, which was at least 50%, suggested that the SiNPs created by this technique were of high purity.

Using the fruit peel from the *Musa paradisiaca*, energy-dispersive X-ray spectroscopy (EDX) demonstrated the chemical makeup of the produced silica nanoparticles. The samples contained a sizable amount of silicon and a weight percentage of oxygen.

Antimicrobial activity

Bacillus subtilis and *Staphylococcus aureus* were used to assess the antibacterial activity of Si nanoparticles on both gram positive and gram negative microorganisms (*Pseudomonas aeruginosa, Escherichia coli*). After being prepared, the sample was determined to be antimicrobial (Table-5). The huge surface area of the Si Nanoparticles contributes to their effective antibacterial capabilities. Reactivity with the gram positive and gram negative bacteria employed in the current study is increased by the smaller particle size. These nanoparticles are so small that they can easily enter bacterial cells, where they trigger an internal inhibitory mechanism. A Si nanoparticles that has entered a bacterial cell causes the cell membrane to be distorted and destroyed, which results in cell death. There is no zone of inhibition in the fluid used to synthesize nanoparticles. Only streptomycin, which was used as the positive control, and the phytosynthesized silica nanoparticles demonstrated a zone of inhibition against both gram positive and gram negative bacteria. Testing the MIC of phytosynthesized silica nanoparticles revealed inhibition at a concentration of 5 g/mL, indicating that these particles can exhibit MIC at extremely low concentrations. The same kinds of outcomes were reported by Gandhi et al., 2021 [37] in their research on the creation of CaO nanoparticles utilizing *Ocimum tenuiflorum* leaf extract.

Agricultural Application of Si Nanoparticles

Since there is a food shortage due to the rising global population. Therefore, it is necessary to double food output by optimizing environmental factors in order to increase crop productivity while using less water in places with a water shortage. With the help of powdered *Musa paradisiaca* fruit peel, the current experiment aims to investigate the impact of seed germination and seedling growth under varied concentrations of phytosynthesized silica nanoparticles. The impact of silica on seed germination and seedling growth of maize at various concentrations was discussed by scientific literature and experimental data acquired by Sun et al., 2021 [38]. His research yielded the following conclusions: silica boosted seed vigour index, shoot length, root length, fresh and dry biomass of crop, and final commercial edible component of maize at lower and optimal concentrations. The significance that phytosynthesized silica nanoparticles play in the growth of tropical and subtropical crops is given very little consideration. In light of this, the current experiment was conducted using silica nanoparticles to examine the effectiveness of silica nanoparticles in promoting plant development in comparison to silica-based fertilisers as a positive control and a negative control with no treatment.

A significant commercial tropical crop growing in a number of arid regions of northern and southern India is Bengal gram (*Cicer arietinum*). It is frequently referred to as "chickpea," and it is a significant pulse crop that plays a significant role in guaranteeing both economic stability and food security. The rate of rainfall and the ground level have dramatically decreased in recent years as a result of the rise in global pollution. Unstable yields and poor seed quality were brought on by the ecological and environmental imbalance.

In the international soil classification system, silicon (Si), which is listed as one of the necessary minerals after nitrogen (N), phosphorus (P), and potassium (K), is defined as resolving water deficiency issues caused in plant bodies and enhancing the synthesis of growth hormones.

Despite soils having a lot of silicon, plants cannot fully absorb all of it [39]. To address the issue of water scarcity, there are now two types of Si fertilizers being employed in conventional agricultural operations. One is soluble in citrate, whereas the other is soluble in water. Citrate-soluble Si fertilizer can be absorbed by crops right away after application; it is insoluble in water but soluble in acid. Citrate-soluble Si is often a byproduct of the high-temperature calcination of ore, fly ash, steel slag, and other materials. However, this sort of fertilizer still contains a lot of harmful byproducts of industrial production because the raw material is mostly regarded as industrial waste and the manufacturing method is quite straightforward. Water-

soluble Si fertilizer is soluble in water and can be used by plants right away. It is created through expensive, complex, and time-consuming high-temperature chemical processes from a tiny amount [40] and the rate at which such fertilizers are absorbed by roots is quite low. Therefore, the goal of the current experiment was to create silica nanoparticles of the nano size using a phytosynthetic process.

To study the impact of phytosynthesized silica nanoparticles prepared by using *Musa paradisiaca* fruit feel powder on Bengal gram (chickpea), growth experiments carried as described in materials methods and results (Table-8) showed that nanoparticle treatments resulted in significant increase in the germination percent, germination rate, germination index and vigor index in comparison with the both positive and negative controls. The maximum values of germination percent (93.78%) and rate (98.06 n/d) were recorded in the T3 treatment and there is increase in growth rate and germination with increased concentration of silica nonoparticle; the maximum average of mean germination time of seeds belonged to silica nanoparticles with T3 treatment (5 days) and minimum values were recorded in negative control. As shown in table-7, silica nanoparticles treated seeds (T1, T2 and T3) promote the germination of chickpea seeds, resulting in GR, GP, GI and VI values that were higher than those in positive control (seeds treated with water soluble silica fertilizer i.e. SiO_2) and negative control (seeds without any treatment). Raskar and Laware 2014, [41] investigated the effects of different concentrations of Zinc Oxide nanoparticle treatments on onion seed germination and early seedling growth. Seed germination and related indices were increased in lower concentrations; however, they were decreased at higher concentrations. According to Karimi et al. (2012) [42], silver nanoparticles did not hinder wheat seed germination. In addition to serving as the traditional fungicide Carboxitiram, silver nanoparticles can protect seeds from fungi. A recent study demonstrates that silica nanoparticles can also provide seed protection from fungi and dangerous bacteria. Similar to silver nanoparticles in that the silica nanoparticles created in this work demonstrated excellent antimicrobial activity. Similar to this, nano-SiO_2 dramatically improved tomato seed germination [43]. The application of silica nanoparticles improved germination, demonstrating the impacts of this nanomaterial. Other investigations that had validated the beneficial benefits of silicon under salinity stress were those by Zuccarini 2008 [44], Lee et al. 2010 [45, 46], Wang et al. 2010 [47], and Wang et al. 2011 [48]. The effects of silver nanoparticles on the germination and growth of eleven common wetland plant species were investigated by Yin et al. in 2012 [49]. They compared the outcomes of two silver nanoparticles: 6-nm gum arabic coated silver nanoparticles and 20-nm poly vinyl pyrrolidine coated silver nanoparticles (PVP-AgNPs) (GA-AgNPs). PVP-AgNP had no effect on germination in the direct exposure studies, whereas 40 mg Ag L21 GA-AgNP exposure dramatically decreased the germination rate of three species and increased the germination rate of *Eupatorium fistulosum* species.

Parveen and Rao 2014, [50] reported that biologically synthesized silver nanoparticles enhanced the percentage of seed germination in *Pennisetum glaucum* but higher concentration of silver nanoparticles decreased the root, shoot and total seedling length in this species. They observed that higher concentration of nanoparticles had adverse effects on plant species. Inhibition of seed germination and root elongation had been found to be highly dependent on both plant type and nano-particle properties.

Karimi et al., 2012 [42] suggested that silver nano-solution coated seeds recorded better germination than the seeds treated with fungicide and concluded to use silver nano-coating instead of using fungicides. Silver Nanoparticles even at the highest concentration did not disturb the germination traits and seedlings growth of *Ricinus communis* [51]. Results of Karimi´s et al., 2012 [42] showed that treating seeds with silver nanoparticles did not reduce germination; it is possible to use this treatment in the agricultural practices. In other words, silver nanoparticles did not affect the seed living process adversely. Results of Haghighi et al., 2012, [52] that studied the effect of nano-Si on tomato seed germination demonstrated that mean germination time had low reduction of 1 and 2 ppm nano-Si application. The experimental results obtained from Alsaeedi et al., 2019 [53], various concentrations of silica nanoparticles enhanced seed germination and growth development of cucumber (*Cucumis sativus*). The rate of 200 mg/L significantly increased final germination %, germination speed, vigor index, and germination index by 28.7, 70.3, 46.7 and 68.8 %, respectively, compared to untreated seeds. However, it reduced the mean germination time by 31.7%. Similarly, 200 mg/L treatement had the highest fresh and dry weight of germinants.

The potential effect of SiO_2 nanoparticles size ranging between 10 – 20 nm and Mo Nanoparticles (< 100 nm) on rice seed germination were studied by Tapan et al., 2013 [54]. Their experiments observed with good germination of seeds with both nanoparticles. SiO_2 nanoparticles had exhibited no toxic effect on rice growth, whereas root growth and elongation were arrested with Mo nanoparticles at higher concentration. In their studies silica nanoparticles enhanced the root length, root volume and dry matter weight of shoot and root of rice crop.

With the increase of concentration (T1, T2 and T3), the growth indices of the seedlings first increased and the positive effects were the most obvious at the higher concentration i.e. T3 treatment (Table-9 & Table-10). Compared with those of the control (NC), the shoot lenght, root lenght, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight under T1 treatment increased by 45.3%, 53.6%, 26%, 10%, 31%, and 8%, under T2 treatment increased by 78.9%, 57%, 29%, 10%, 35% and 8

% and under T3 treatment increased by 99%, 62%, 31%, 15%, 35% and 13% respectively at 15 days of crop age. The shoot height, root height, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight were compared with control (NC) at 30^{th} day of crop age and T1 treatment increased by 62.5%, 60.4%, 28%, 12%, 23% and 10%, with T2 treatment increased by 90%, 62%, 35%, 13%, 27% and 12% and with T3 treatment increased by 100%, 75.4%, 40%, 20%, 40% and 30% respectively.

Duncan testing revealed that the T3 treatment had the longest root and shoot lengths (22.78 and 35.50 at seedling age 30 days). Furthermore, the control treatment had the shortest root and shoot lengths (NC). As the concentration of silica nanoparticles increased, so did the length of the roots and shoots. Sunflower germination and early growth features were explored by Vashisth and Nagarajan in 2010 [55], who also evaluated the impacts of ZnO nanoparticle treatments at various concentrations and reported comparable findings. They claimed that ZnO nanoparticles had a positive impact on the development of roots and shoots.

Nano-structured silicon dioxide's effects on Changbai larch (*Larix olgensis*) seedling growth were studied by Bao-shan et al. in 2004 [56]. They noticed that the silicon dioxide-treated Larix seedlings' quality and growth were enhanced. The largest seedling length, root collar diameter, root length, and number of lateral roots were seen at 500 µL/L. Results showed that T3 treatment produced the highest means of seedling fresh and dry weights for silica nanoparticles, while both positive and negative control treatments produced the lowest means.

The findings of Zhu et al., 2008 [57] on *Phaseolus vulgaris* demonstrated that varied concentrations of ZnO nanoparticles had an impact on shoot length, fresh and dry weights of shoots, and dry shoot weight was substantially different among the treatments. The control group, however, had the lowest fresh and dry weights. However, in contrast to the other concentrations and the control, the highest shoot length was found at 20 mg. Additionally, Haghighi et al. (2012) [52] stated that silica nanoparticles had significant benefits in improving salt stress on tomato seed germination. Silica nanoparticles at a concentration of 25 ppm significantly improved germination traits as germination rate, root length, and dry weight. The similar type of results observed with Gandhi et al., 2018 when carried experiment with lead nanoparticles[31] and copper nanoparticles [32]. The (Figure-11), which are all indicators of plant stress. The findings unmistakably demonstrate that the Si phytosynthesized nanoparticles utilized in this investigation had no harmful or adverse effects on growth metrics, but instead promoted seedling growth. The levels of toxicity were discovered to have decreased. These findings on $CaCO_3$ Nanoparticles' impact on seed germination and seedling growth on *Vigna mungo* were also made for Yugandhar & Savithramma in 2013 [58]. The obtained results also show that the utilization of Si phytosynthesized Nanoparticles prepared from fruit peel of *Musa paradisiaca* will give an economical yield to farmers especially in water scarced areas to enhance the growth rate of Bengal gram (*Cicer arietinum*).

The seedling growth analysis parameters of Bengal gram (*Cicer arietinum* L.) seedlings, including relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), leaf weight ratio (LWR), specific leaf area (SLA), specific leaf weight (SLW), and leaf area duration (LAD), were examined. It was found that Si phytosynthesized nanoparticles showed an increase in growth with increasing concentration and crop growth periods, compared to Gandhi et al., 2020 [13] conducted studies with stress parameters impacting CaO nanoparticles to explain similar reports.

Total carbohydrates, proteins, photosynthetic pigments, and POD enzyme activity were biochemical parameters that were assessed in relation to all treatments. The results are given in tables 11 and 12. The concentration of Si phytosynthesized nanoparticles was found to increase along with all biological contents. By boosting effects on seedling growth, some research focused on the biological creation of nanoparticles. The biological production of silica nanoparticles and their stimulatory effects on plants have only been the subject of a very small number of investigations to date. The purpose of the current study is to manufacture silica nanoparticles and determine how they affect Bengal gram seed germination and seedling growth (*Cicer arietinum* L.). Results demonstrated that silica nanoparticles can be used to chickpea (*Cicer arietinum* L.) crops as an inorganic fertilizer.

V. CONCLUSION AND FUTURE SCOPE

The application of phytosynthesized silica by employing fruit peel powder of *Musa paradisiaca* has greatly improved the seed germination potential, according to the current study's findings in general. All of the seedlings' early growth traits (*Cicer arietinum* L.) were enhanced by the application of silica. The findings demonstrated that, in comparison to silica nanoparticle treatments of T1, T2, and control treatments, i.e. positive and negative controls, nano-silica with T3 treatments had a greater impact on the germination of (*Cicer arietinum* L.). Overall, silica nanoparticles with T3 treatment showed greater values of seed germination features, indicating that higher Si nanoparticle concentrations are essential for crop growth. The biological parameters (carbohydrates, proteins, POD, and photosynthetic pigments) have grown together with the concentration of silica nanoparticles. Because most microorganisms are sensitive to nanoparticles, several studies are needed to manufacture nanoparticles in smaller sizes by displaying microbial activity at the open field level. The findings of the current study demonstrated that the production of Si nanoparticles may be done quickly, cheaply, and environmentally friendly using green synthesis techniques. This will be used in a variety of industrial, medical, and agricultural development projects.

Figures and Tables

Figure-1: Scientific classification of banana & peel powder preparation procedure

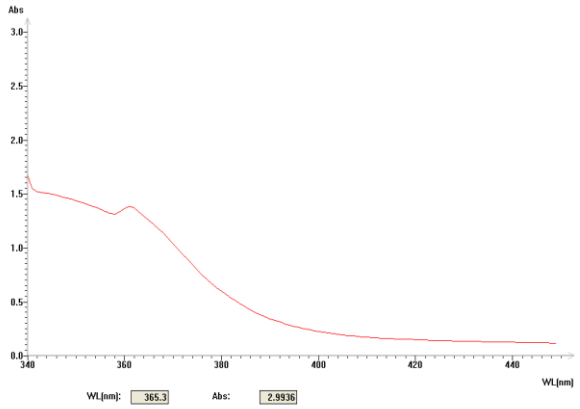


Figure-2: Silica Nanoparticles are synthesized by UV spectrum by using *Musa paradisiaca* fruit peel powder

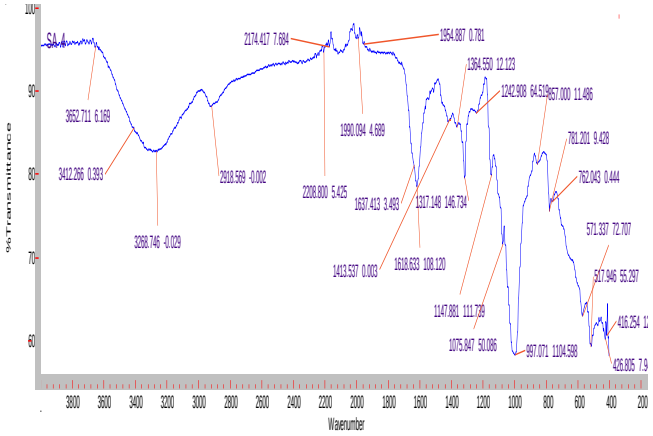


Figure-3: FTIR spectra of dried banana peel powder used for Si nanoparticles synthesis

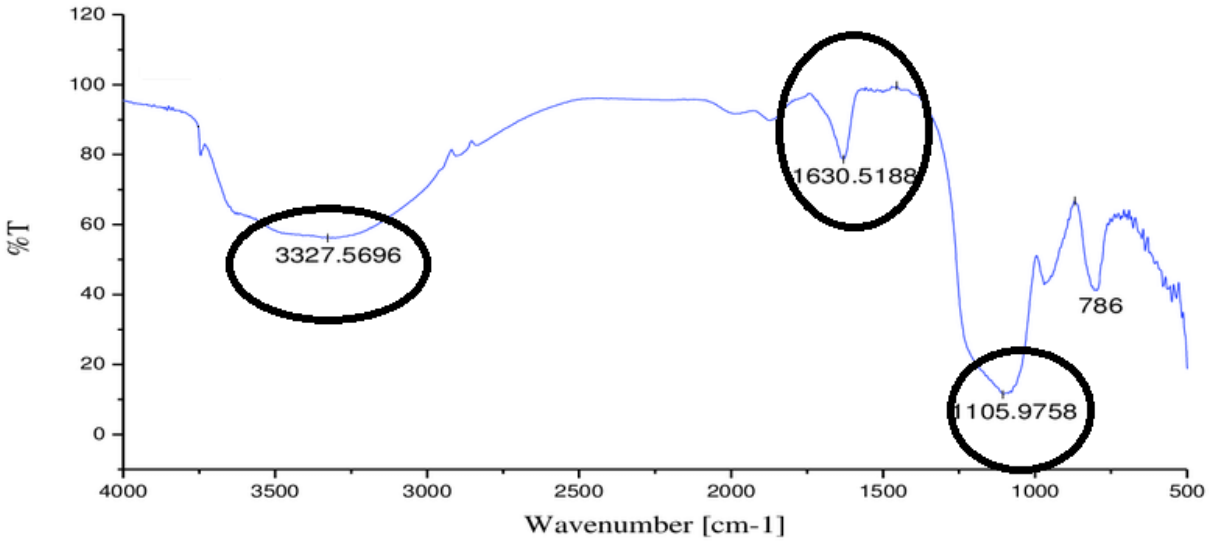


Figure-4: FTIR spectra of phytosynthesized Si nanoparticles from dried banana peel powder.

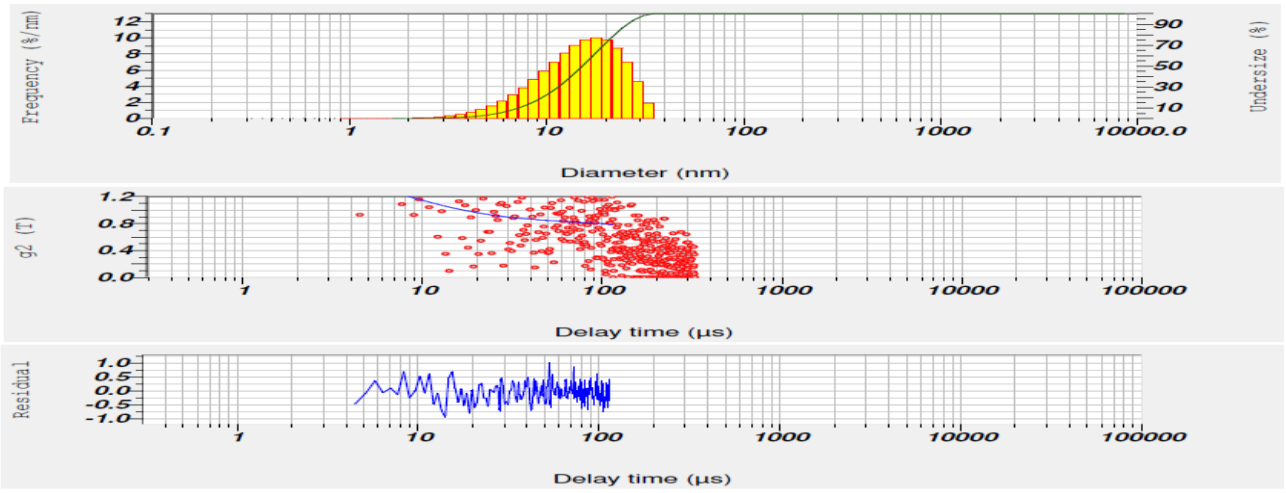
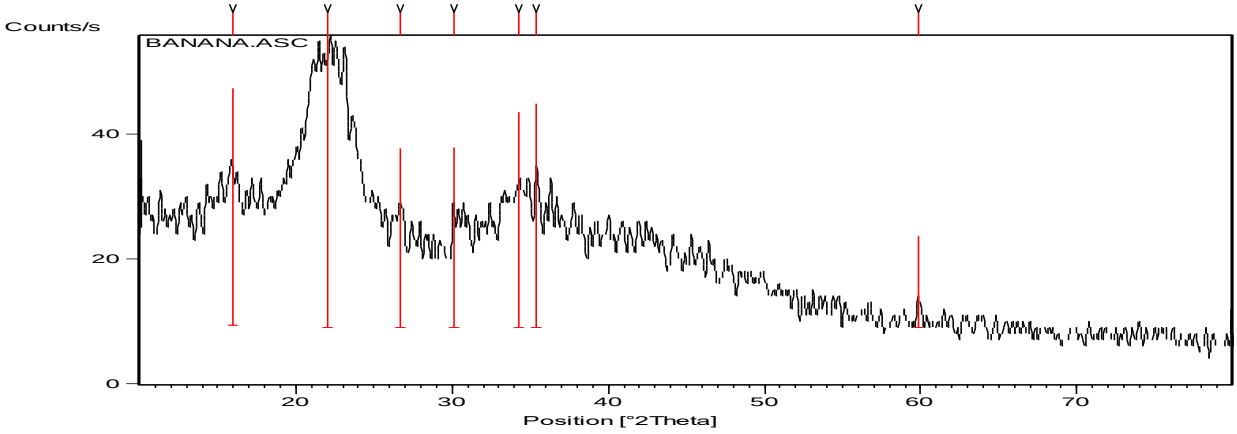


Figure-5: DLS results for silica nanoparticles synthesized by using *Musa paradisiaca* fruit peel powder



Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
15.9300	38.14	0.0200	5.56359	63.59
22.0813	59.97	1.2513	4.02566	100.00
26.6587	28.77	0.3973	3.34393	47.98
30.0900	28.95	0.0200	2.96997	48.27
34.2900	34.64	0.6257	2.61520	57.76
35.4100	35.94	0.0200	2.53501	59.93
59.8100	14.69	0.1060	1.54632	24.49

Figure-6: XRD results for phytosynthesized Si nanoparticles using *Musa paradisiaca* fruit peel powder.

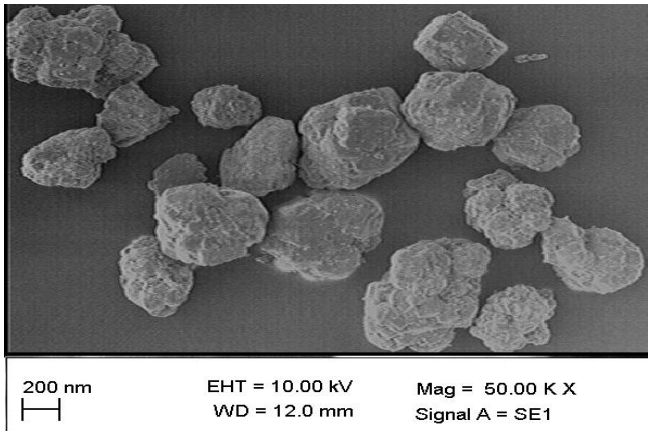


Figure-7: SEM results for phytosynthesized Si nanoparticles using *Musa paradisiaca* fruit peel powder.

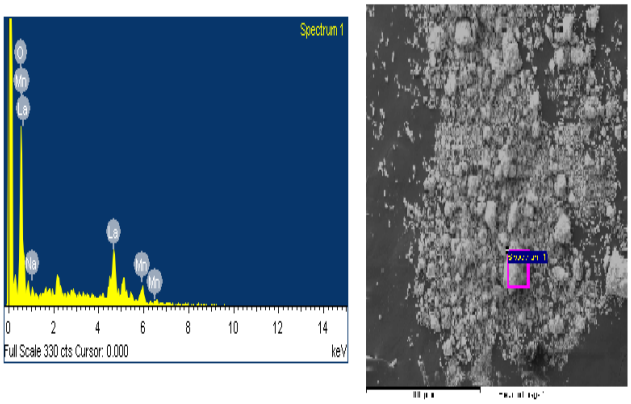


Figure-8: EDX results for phytosynthesized Si nanoparticles using *Musa paradisiaca* fruit peel powder.

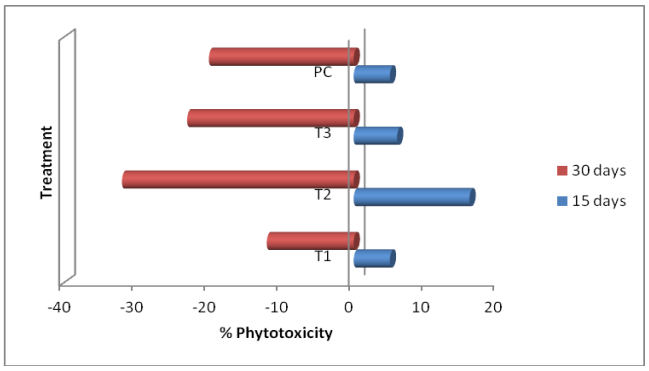


Figure-9: % phytotoxicy of phytosynthesized SiNP on seedling growrth of *Cicer arietinum.*

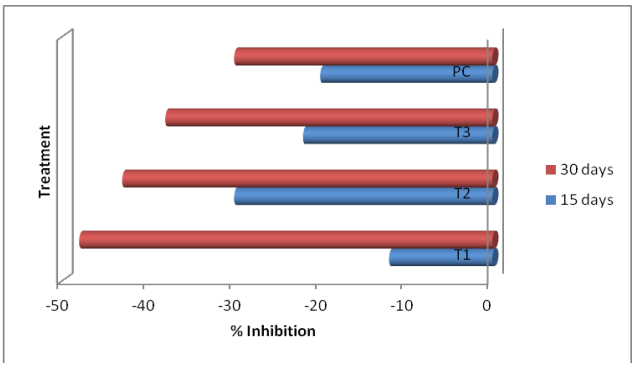


Figure-10: % inhibition of phytosynthesized SiNP on seedling growrth of *Cicer arietinum.*

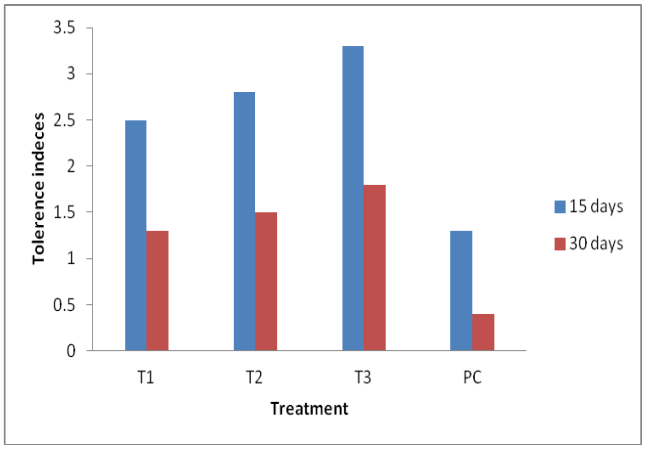


Figure-11: Tolerence indeces of phytosynthesized SiNP on seedling growrth of *Cicer arietinum*

Table-1: Simple peak index

Peak position (2θ)	1000 X $\sin^2$ θ	1000 X $\sin^2$θ/46
15.93	20	0.434
22.08	38	0.826
26.65	54	1.173
30.09	69	1.501
34.29	88	1.913
35.41	94	2.043
59.81	251	5.450

Table-2: Peak indexing from d-spacing

Peak position (2θ)	d-spacing	1000/d2	(1000/d2)/77.32
15.93	5.56359	89.9	1.16
22.08	4.02566	124.2	1.60
26.65	3.34393	149.7	1.93
30.09	2.96997	168.6	2.18
34.29	2.61520	191.2	2.47
35.41	2.53501	197.2	2.55
59.81	1.54632	323.6	4.18

Table-3: Particle size calculations

Peak position (2θ)	Particle Size (nm)
15.93	7
22.08	11.2
26.65	35
30.09	7.2
34.29	23.1
35.41	7.3
59.81	15.09

Table –4: Elemental analysis of EDX

S.No	Element	Weight (%)	Atomic (%)
01	$CaCO_3$ K	54.25	61.60
02	SiO_2 K	43.62	37.19
04	Na K	0.52	0.25
05	Si K	1.60	0.95

Table-5: Anti bacterial activity (Zone of inhibition) of phytosynthesized silica nanoparticles

S.No	Organism	Zone of inhibition (mM)
01	*E. coli*	14.6
02	*Pseudomonas aeruginosa*	13.2
03	*Bacillus subtilis*	11.4
04	*Staphylococcus aureus*	8.2

Table-6: Water quality parameters includes the amount of water used for irrigation)

S.No	Parameter	Analyzed value
01	pH	6.9 to 7.2
02	Electrical Conductivity	130.00 µmhos/cm
03	Salt content	0.03 ppt
O4	DO	9.72 mg/L
05	NO_3^- N	0.84 mg/L
06	NO_2^-	0.02 mg/L

07	Ca + Mg	84 mg/L
08	SO_4^-	23 mg/L
09	Cl^-	2.7 mg/L
10	Ca	17 mg/L
11	Mg	8 mg/L

Table-7: Soil quality parameters include 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-8: Effects of the phytosynthesized Si nanoparticles using banana peer powder on germination indices of Bengal gram (Chickpea).

S.No	Treatment	GR (%)	GP (%)	GI	VI
01	Negative Control	82.00 ± 2.00	44.00 ±2.00	11.78 ± 0.66	9.36 ± 0.77
02	Positive Control	93.63 ± 0.60	58.00 ± 3.33	15.75 ± 0.36	16.97 ± 0.46
03	T1	96.01 ± 0.57	73.37 ± 0.53	19.99 ± 0.82	17.85 ± 1.24
04	T2	97.84 ± 1.87	81.11 ± 3.85	23.11 ± 0.86	22.43 ± 1.18
05	T3	98.06 ± 0.67	93.78 ± 1.92	39.01 ± 0.70	26.67 ± 0.52

The germination, growth indices values represent the mean ± SE (n = 5). Values obtained by ANOVA concluded that there is significantly different impact in germination index between controls and treatments at $P < 0.05$.

Table-9: Effects of phytosynthesized Si nanoparticles using banana peel powder on seedling growth of chickpea at t= 15 days.

S.No	Treatment	Shoot length (Cm)	Root Length (Cm)	Shoot Fresh Weight(gm)	Root Fresh Weight(gm)	Shoot Dry Weight (gm)	Root Dry Weight (gm)
01	Negative Control	12.30 ± 0.46	4.53 ±0.31	0.28 ± 0.01	0.12 ± 0.01	0.17 ± 0.01	0.07 ± 0.01
02	Positive Control	16.50 ± 0.31	5.89 ± 0.23	0.32 ± 0.01	0.17 ± 0.01	0.24 ± 0.01	0.10 ± 0.01
03	T1	16.83 ± 2.32	9.89 ± 0.32	0.54 ± 0.02	0.22 ± 0.01	0.48 ± 0.01	0.15 ± 0.01
04	T2	20.19 ± 0.01	10.23 ± 0.39	0.57 ± 0.01	0.22 ± 0.01	0.52 ± 0.01	0.14 ± 0.01
05	T3	22.78 ± 0.06	10.58 ± 0.23	0.59 ± 0.01	0.24 ± 0.01	0.52 ± 0.01	0.20 ± 0.01

The above values show that the mean ± SE (n = 4) are obtained by ANOVA concluded that there is significant difference in germination index between controls and treatments at < 0.05.

Table-10: Influence of phytosynthesized nanoparticles by using banana peel powder on seedling growth of chickpea at t= 30 days.

S.No	Treatment	Shoot length (Cm)	Root Length (Cm)	Shoot Fresh Weight(gm)	Root Fresh Weight(gm)	Shoot Dry Weight (gm)	Root Dry Weight (gm)
01	Negative Control	17.23 ± 0.35	6.58 ± 0.46	0.40 ± 0.02	0.13 ±0.01	0.36 ± 0.01	0.09 ± 0.01
02	Positive Control	22.63 ±0.25	9.55 ± 0.12	0.48 ± 0.01	0.18 ±0.01	0.40 ± 0.01	0.12 ± 0.01
03	T1	23.48 ± 0.01	12.62 ± 0.21	0.68 ± 0.01	0.25 ±0.01	0.59 ± 0.01	0.19 ± 0.01
04	T2	28.34 ± 0.05	12.78 ± 0.58	0.75 ± 0.01	0.26 ±0.01	0.63 ± 0.01	0.20 ± 0.01
05	T3	35.50 ± 0.06	14.12 ± 0.01	0.80 ± 0.01	0.32 ±0.01	0.76 ± 0.01	0.25 ± 0.01

The above values show that the mean ± SE (n = 4) are obtained by ANOVA concluded that there is significantly different impact in germination index between controls and treatments at $P < 0.05$.

Table-11: Effects of phytosynthesized Si on the photosynthetic pigment contents of Bengal gram/chickpea.

S.No	Age of the crop	Treatment	Chl-a	Chl-b	Chl-a +b	Cartonoides
01		Negative Control	4.29 ± 0.06	1.81 ± 0.05	6.10 ± 0.01	0.36 ± 0.02

02	15 days	Positive Control	5.33 ± 0.06	2.87 ± 0.07	8.20 ± 0.01	0.44 ± 0.01
03		T1	6.25 ± 0.02	3.01 ± 0.07	9.26 ± 0.01	1.45 ± 0.03
04		T2	7.22 ± 0.01	3.26 ± 0.01	10.48 ± 0.01	1.52 ± 0.01
05		T3	7.47 ± 0.01	3.55 ± 0.06	11.02 ± 0.01	1.58 ± 0.05
01	30 days	Negative Control	5.26 ± 0.03	2.49 ± 0.05	7.75 ± 0.01	1.30 ± 0.02
02		Positive Control	5.60 ± 0.03	2.98 ± 0.08	8.58 ± 0.01	1.46 ± 0.01
03		T1	6.38 ± 0.02	3.15 ± 0.01	9.53 ± 0.01	1.93 ± 0.03
04		T2	7.69 ± 0.01	3.86 ± 0.01	11.55 ± 0.01	1.99 ± 0.01
05		T3	7.82 ± 0.01	3.99 ± 0.02	11.81 ± 0.01	1.99 ± 0.01

The above values show that the mean ± SE (n = 4) are obtained by ANOVA concluded that there is significantly different impact in germination index between controls and treatments at $P < 0.05$.

Table-12: Effects of phytosynthesized Si nanoparticles on the contents of osmolytes and enzymes (Total carbohydrates, protein and peroxidase enzyme activity).

.S.No	Age of the crop	Treatment	Total Carbohydrates	Total Proteins	POD enzyme activity
01	15 days	Negative Control	4.06 ± 0.09	2.23 ± 0.04	11.59 ± 0.09
02		Positive Control	5.03 ± 0.03	2.97 ± 0.06	12.67 ± 0.09
03		T1	5.44 ± 0.07	3.53 ± 0.08	15.45 ± 0.08
04		T2	5.78 ± 0.07	3.67 ± 0.02	20.54 ± 0.01
05		T3	5.99 ± 0.03	3.97 ± 0.09	33.75 ± 0.03
01	30 days	Negative Control	4.33 ± 0.03	2.69 ± 0.08	14.22 ± 0.01
02		Positive Control	6.44 ± 0.03	3.26 ± 0.04	15.32 ± 0.03
03		T1	6.63 ± 0.07	4.22 ± 0.01	19.67 ± 0.03
04		T2	6.90 ± 0.07	4.52 ± 0.06	26.77 ± 0.09
05		T3	7.96 ± 0.03	5.23 ± 0.01	39. 56 ± 0.08

VI. ACKNOWLEDGMENT

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