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Rhodobacter sp. and Nano-silicon Dioxide enhanced phosphate solubilization

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ABSTRACT

Phosphate-solubilizing bacteria (PSB) are significant plant growth-promoting rhizobacteria that enhance the growth of plants by solubilizing insoluble phosphates and facilitating their uptake. *Rhodobacter* sp. is one of the PSBs identified from the rhizospheric soil of *Tectona grandis*, and it can solubilize up to 80% of phosphates in in-vitro conditions. In the present study, nanoparticles along with PSB were given as treatments. The excellent nature of silica to uptake nutrients and stress tolerance makes it different from other nanoparticles. Plant based green synthesis of Silicon nanoparticles from *Gigantochloa nigrociliata* leaves developed the spherical shape with 349.7 nm in Scanning Electron Microscopy (SEM), peak at 1033.85cm^{-1} in Fourier Transform Infrared Spectroscopy (FTIR), amorphous nature and surface area of 312.46 m^2/g in X-ray diffraction (XRD) was recorded. The combination of both PSB and SiO2 showed the synergistic activity of 335×10^{-1} Colony Forming Units (CFU). The present study shows that the mixture of 0.005% of SiO_2 and 5 gm. (50%) of *Rhodobacter* sp. acts as a better nano-biofertilizer. This results in the growth and improvement of *Zea mays*. This method provides an alternative to GMO crops, fertilizers, and traditional insecticides.

Keywords: Nano silicon dioxide; phosphate solubilizing bacteria; Bio fertilization

INTRODUCTION

The reckless use of agrochemicals has presented a significant threat to both the environment and public health. Fertilizers, which are chemical substances that promote plant growth, have different impacts depending on whether they are organic or chemical and whether they are applied through soil or foliage. To address the challenges faced by farmers, the use of chemical fertilizers has been suggested. This reliance on chemical fertilizers has increased as a strategy to overcome these challenges and achieve higher crop yields (Glick, 2012). Chemical fertilizers tend to be more effective initially, but their effectiveness diminishes over time. The adverse effects of these fertilizers have led to a deteriorating environmental condition. Issues such as nutrient depletion, water scarcity, and soil degradation are some of the concerns associated with the use of chemical fertilizers. The environmental consequences of continuous use of chemical fertilizers, especially those high in phosphorus, nitrogen, and potassium, are well recognized (Adesemeye and Kloepper, 2009). In order to enhance agricultural productivity in a sustainable manner, innovative technologies need to be integrated with improved farming practices. Essential minerals are largely absent in contemporary agricultural practices without the use of artificial fertilizers, leading to detrimental effects on the ecosystem (Garg *et al.,* 2023).

To address this issue, it is crucial to introduce beneficial microorganisms such as nitrogen-fixing bacteria, mycorrhizal fungi, and phosphobacteria (Karthikeyan *et al.,* 2009). Phosphorus, a vital macronutrient that limits growth, plays a crucial role in optimal plant development, particularly in tropical regions where its availability in the soil is limited. Among the most effective phosphate-solubilizing bacteria in soil are bacteria from the genera *Pseudomonas, Bacillus*, *Rhizobium*, and *Enterobacte*r. These Phosphate-Solubilizing Microorganisms (PSMs) can release various organic acids as byproducts of their microbial metabolism, primarily through oxidative respiration or fermentation when glucose is used as the carbon source (Alam *et al.,* 2002; Sathyaprakash *et al.,* 2017). Phosphobacteria generally contribute to plant growth and enhancement by solubilizing insoluble phosphates, making it easier for plants to absorb the nutrient. Karunakaran *et al.* (2013) suggested that the use of nano silica can stimulate the population of PGPRs in soil by improving soil pH and promoting the germination of maize seeds.

Khati *et al.* (2019a, b) found that the application of nano zeolite enhances the growth of PGPRs. A previous study demonstrated that the application of a complex Nano fertilizer increased the growth parameters of plants (Al-Jibouri *et al.,* 2023). However, a comprehensive investigation into the reciprocal interactions between bio inoculants and nanoparticles is necessary to facilitate exploration within the context of sustainable cultivation strategies. The utilization of nanobiofertilizers in the field of agriculture initiates a fresh era in the sustainable cultivation of crops (Akthar *et al.,* 2022). The study at hand specifically selected Silicon nanoparticles as the nanomaterial to enhance phosphate adsorption by plants. By utilizing SiO_2 in conjunction with *Rhodobacter* sp., the growth of *Zea mays* was targeted for improvement within a nursery setting. The main objective of this investigation is to assess the impact of nanosilicon dioxide combined with PSB on various plant growth parameters, such as plant height, chlorophyll content, carotenoid levels, protein concentration, flavonoid content, and soil enzyme analysis throughout the maize cultivation process.

MATERIALS AND METHODS

Synthesis of Silicon Nanoparticle:

The ***Gigantichloa nigrociliata*** leaves were obtained from the Silviculture nursery, IFGTB Coimbatore, India. After being fully grown, the leaves were carefully washed with running tap water. Following this, the leaves were allowed to dry naturally for a period of 2 days. Once they were completely dry, the desiccated foliage was finely ground using an analytical mill. The resulting powder was then subjected to further drying on a hot plate, at a temperature of 75°C, until it was completely devoid of any moisture. Subsequently, 10 grams of the powder were combined with 250 ml of HNO_3 and left to react for duration of 12 hours, using a magnetic stirrer. The mixture was then filtered using Whatman No.1 filter paper in order to separate any impurities. The purified solution was dissolved in 1 N NaOH and maintained at a constant temperature of 64°C, while being stirred magnetically for a period of 2 hours. The filtered solution was once again passed through a Whatman No.1 filter paper to ensure the removal of any remaining impurities. The filtered material was then treated with 1 N H_2SO_4 until a white-colored product was formed. The resulting Na_2SiO_3, however, was still in an unpurified state. To rectify this, the powder was placed in a hot air oven and subjected to a temperature of 65°C for a duration of 1 hour. This procedure successfully purified the Na2SiO3, resulting in a refined product (Sethy *et al.,* 2019).

Characterization:

The identification of nanoparticles can be done by the characterization technique. The SEM, XRD, FTIR, and UV – Visible spectroscopy have been done. Scanning electron microscopy has been done to the surface analysis of the nanoparticle as well as to know the structure of the nanoparticle. The X-ray diffraction is done to detect the size using Bragg's law and the nature of the particle. The functional group can be identified using the Fourier transform infrared spectroscopy. The concentration of the samples per ml can be identified using UV-visible spectroscopy.

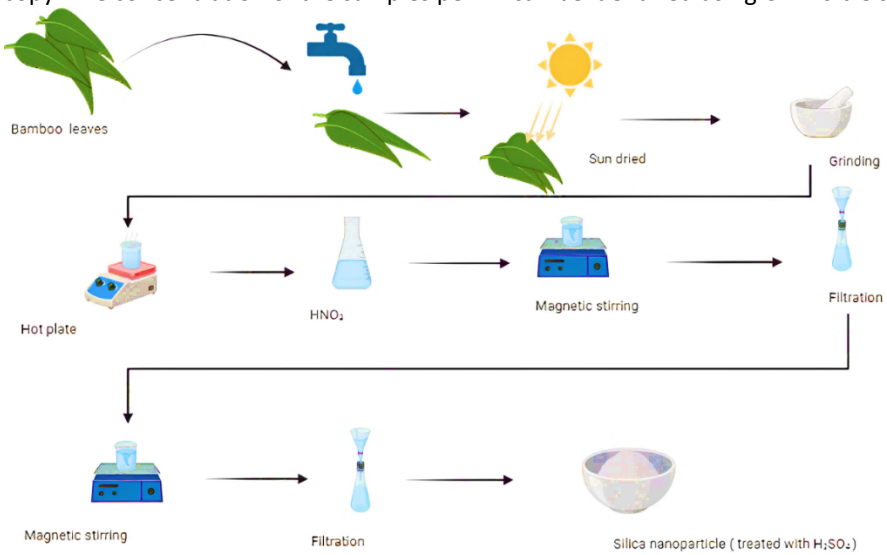


Fig. 1. The pictorial representation of green synthesis of silicon nanoparticle through acid extraction followed by alkali precipitation (Bio RENDER)

Isolation of PSB:

The soil sample has been collected from the rhizospheric region of the *Tectona grandis*, silviculture nursery, IFGTB, Coimbatore, India. The soil was kept for air drying and then the physio-chemical characteristics of the soil were analyzed. The sample was subjected to pure culture techniques. The isolated organism is spot inoculated on the Pikovaskaya's media with the supplication of tri-calcium phosphate to identify the solubilizing efficiency.

The phosphate solubilizing efficiency can be calculated by,

E = Diameter of Bacterial growth **/** Diameter of Clearing Zone × 100

. Where E is phosphate solubilization efficiency.

PGPR activity :

In vivo Analysis:

Production of IAA (Indole Acetic Acid):

The bacterial cultures were inoculated in Luria Bertani media with the supplication of tryptophan as a precursor. The culture was incubated in an orbital shaker at 250 rpm at 30^0c for 3-5 days. The fully grown cultures were centrifuged at 10000 g for ten minutes. Combine 4 ml of Salkowski reagent (50 ml; 35% $HClO_4$&1 ml 0.5 M $FeCl_3$) with 2 ml of supernatant. The pink color indicates a positive result. (Ehmann,1977)

Organic acid production:

The test organism was inoculated at a Minimal Salt medium (MM9 broth). MM9 broth composed of 133 ml of minimal media added to 867 ml of sterile water. After 2-3 days of incubation at 30^0c, add the methyl red indicator into the media. The formation of pink color in the media results positive result. (Bouizgarne et al.,2013)

Ammonia production:

Ten ml of peptone water were used to inoculate recently formed cultures, and they were then cultured for 48 hours at 30^0c. Add 0.5 ml of Nessler's reagent to each tube following a 48 hour incubation period. The color change from brown to yellow indicates a positive result (Cappuccino and Sherman, 1992).

Ex vivo Analysis:

Screening for Hydrogen Cyanide Production:

The test organism was inoculated on the nutrient sucrose media plates supplemented with 4.4 g/liter of glycine. Up to the plate's top place the Whatman No:1 filter paper which is already soaked with 2% Sodium Carbonate ($NaCO_3$) in 0.5% Picric acid solution. Seal it with a parafilm and incubate it for 4 days at 30^0c. The indication of color change from deep yellow to brown or orange represents the positive result (Lorck, 1948).

ACC Deaminase activity:

The test organism was inoculated in the Dworkin Foster Salts medium.(Di hydrogen phosphate-2 gm, Di sodium hydrogen phosphate- 6gm, Magnesium Sulphate heptahydrate- 0.2gm, Glucose- 2 gm, Gluconic acid -2 gm, Citric acid- 2 gm. Trace elements Ferrous Sulphate heptahydrate -1mg, Boric acid10- mg, Manganese Sulphate monohydrate- 11.19 mg, Zinc sulfate heptahydrate- 124.6 mg, Copper sulfate pentahydrate -78.22mg, Molybdenum Trioxide- 10 mg, ACC -3Mm). The plates were inoculated at 28^0c for 3 days. The growth in the plate indicates the ACC deaminase activity.

Siderophore Production:

Before the test, to avoid the contamination of iron from the glassware they should soak in 2N HCl (Hydrochloric acid) for 24 hours. The isolates should be inoculated on CAS blue agar plates. After the incubation for 5-7 days at 28^0c the yellow-orange hollow zone around the colonies can be seen. It indicates a positive result for the Siderophore hormone (Schwyn and Neilands, 1987).

Synergistic activity:

The synergism between the phosphate solubilizing bacteria and the synthesized nanoparticle has been analyzed qualitatively by the dual culture technique, where the culture and nano-impregnated disc have been plated on the plate. After incubation, it shows mutually interrelated with each other.

Quantitatively the synergism has been analyzed using the Time Kill Kinetic assay. The test organism was forced to grow on the nanoparticle-supplemented broth and the microbial count was done using the spread plate technique.

Pot experiment details:

Healthy seeds were taken and washed with tap water. The washed seeds were kept overnight in 1% carboxymethyl cellulose and 50% bacterial culture (bacterial culture was already mixed with 0.005% $nSiO_2$). Then the consortium was held for 15 minutes at 70 rpm in a rotary shaker. Based on the randomized block design 3 replicates were kept for each treatment.

Inoculation of the PSB strain and Nano compound:

Different treatments were given to the plants to identify the best result. The treatment was given as follows (table 1).

Table 1. The number and the corresponding amount of the treatments given to plants.

Treatment		Amount applied
T1	PSB (*Rhodobacter sp*.)	5 ml (50%)
T2	NANO + PSB	0.05% +5 ml
T3	NANO ($nSiO_2$)	0.05%
T4	CHEMICAL	2.5 gm.
T5	CONTROL	

ASSESSMENT OF PLANT GROWTH TRAITS:

Germination percentage:

The germination percentage of each treatment was computed with the formula,

Germination percentage % = Number of seedlings germinated / total number of seeds ×100

Agronomical and biochemical traits:

The height of the plant, girth of the stump (meter scale), and number of leaves were measured. The number of observations were taken every 15 days of germination.

Chlorophyll Estimation:

Fresh leaves were gathered, chopped, and mixed with DMSO (dimethyl sulfoxide) after that, the mixture was kept at 60 ^{0}c in a waterbath for 3 hours, or until it became colorless. Subsequently, it was left at room temperature for one hour. The absorption was analyzed using wavelengths of 663 nm and 665 nm in the spectrophotometer (Hiscox and Israelstam, 1979).

Estimation of carotenoid:

Fresh leaves were collected, cut into pieces, and mixed with DMSO (dimethyl sulfoxide). The mixture was incubated in a water bath for 3 hours at 60°C until it turned colorless. It was then kept at room temperature for one hour. The sample analyzed using a spectrophotometer at 480 nm wavelength (Kirk and Allen, 1965)

Total sugar estimation:

Dried leaves were collected. The leaves were ground using a mortar and pestle by 3% of 80% ethyl alcohol. The mixture wasthen boiled in a water bath. Following cooling, the mixture was centrifuged for 15 minutes at 1000 rpm. The supernatant was taken and made up to the final volume using 80% ethyl alcohol. Four ml of the ice cold anthrone reagent were combined with 1 ml of the extract. Mixed well and keep it in the water bath for 10 minutes. The sample was measured at 680 nm using a spectrophotometer. (Glucose std;10-100 µg/ml) (Dubois *et al.,* 1956).

Estimation of protein:

A gram of newly picked leaves was taken and crushed using a mortar and pestle for 20 minutes. Five ml of 0.2 M Tris–Cl 9 (p^H-8) was added to the slurry. Centrifuge it for 20 minutes at 10000 rpm at 4^{0}c. The supernatant was transferred to new tubes and stored at 4^{0}c. The 20 µl of extract was mixed with 300 µl of extraction buffer. After 10 minutes, incorporate 3 ml of Coomassie Brilliant Blue into the mixture. Incubated the solution for 5 minutes at 37^{0}c.Using a spectrophotometer set at 595 nm, the sample was examined (BSA std: 10-100µg/ml). Bradford et al.,1976.

Soil enzyme activities:

Dehydrogenase activity:

In a 150 ml flask, five grams of soil sample and 5 ml of triphenyltetrazolium chloride were combined. For 8 hrs of 120 rpm and 37 ^{0}C, the mixture was stored in an incubator shaker. Following a 25 ml acetone mixture, the material was centrifuged for 10 minutes at 4500 rpm. Whatman No.1 filter paper was used to filter the supernatant. At 485 nm, the extract was measured (Casida *et al.,*1964)

Alkaline phosphatase activity:

250 µl of toluene, 4 ml of buffer, and 1 ml of P-nitro phenyl phosphate were mixed with one gram of soil. The mixture was incubated at 37 ^{0}c for 2 hours. The extract was mixed with 1 ml of calcium chloride and 4 ml of Tris buffer following the 2-hour incubation. Following the observation of a color shift, the filter paper was used to filter the solution. At 400 nm, the absorption was observed (Tabatabai and Bremner, 1969).

Invertase activity:

A 2 gm sample of fresh soil was taken and combined with phosphate buffer and sucrose solution. The solution was mixed with five drops of toluene. For a full day, the combination was shaken by orbit. After adding glucose and 3,5-dinitrosalicylic acid to the mixture, the absorbance was measured at a wavelength of 508 nm (Frankeberger and Johanson., 1983).

ß glucosidase activity:

Toluene was applied to 0.5 grams of fresh soil, and it was then left undisturbed for 15 minutes. The combination was then given a one-hour incubation at 37^{0}c after the addition of p-nitrophenyl ß-D-glucoside solution. Calcium chloride and tris solution were added to the mixture following the incubation period. A spectrophotometer set to 410 nm wavelength was then used to analyze the material. (Chang *et al.,* 2011).

RESULTS

Synthesis of silicon nanoparticle:

The particle size of prepared nanoparticles through the peptization process by using different electrolytes like nitric acid HCl or H_2SO_4. The synthesized white color silicon nanoparticles have undergone the calcination process to get the purest form. The calcination process was done in a Hot air oven at 600 ^{0}c for 3 hr.

ACID	Particle size
Hydrochloric Acid	349.7 nm

Characterization:

UV-Visible Spectroscopy

In the present green synthesis protocols, the silicon dioxide nanoparticles have been produced from *Gigantichloa nigrociliata* using acid extraction followed by alkali precipitation. The white color powder produced at the final stage indicates the synthesis of silicon nanoparticles. The peaks were found to shift toward a lower wavelength as the particle size reduced from 500 nm. The intensity of the peaks was found slightly similar.

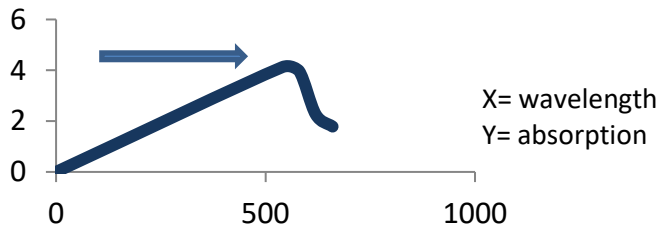
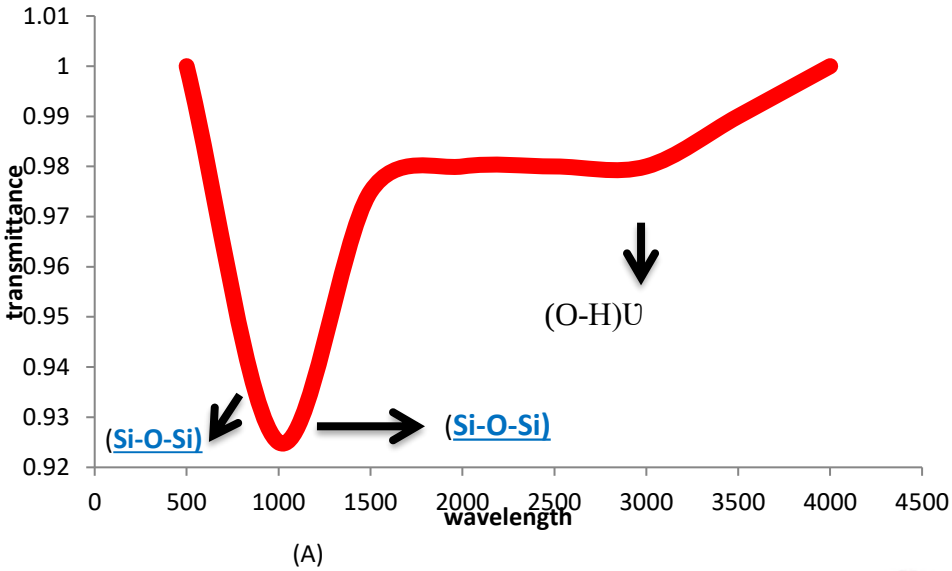


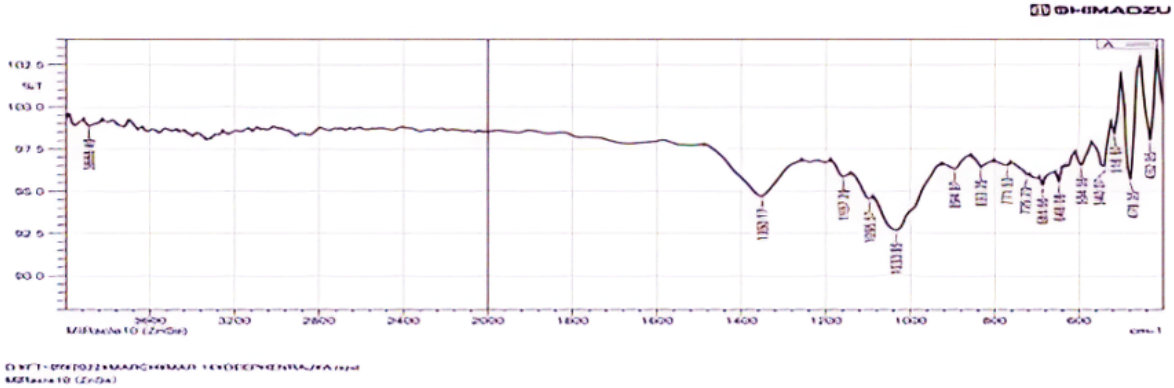
Fig 2. (a)UV-Visible spectra of synthesized SiO2. The UV spectra of the synthesized nanoparticles showed the absorption peak at 540 nm

FT-IR Analysis:

The silicon nanoparticles that were synthesized underwent analysis through FTIR spectroscopy. The FTIR spectrum was utilized to examine the functional groups of these synthesized particles. The spectral data was collected within the range of 4000-5000 cm^{-1}. Information regarding the functional groups present in the synthesized nanoparticle can be found in Figure 2(b). Specifically, in Figure B, the peak observed at 478.35 cm^{-1} corresponds to the bending vibrations of the Si-O-Si component. Additionally, the peak at 833.25 cm^{-1} indicates the presence of Si-O bending vibrations. Furthermore, the broad peak at 1033.085 cm^{-1} is associated with the asymmetric Si-O-Si bonding structure between the O and Si atoms. Lastly, the peak at 3888.49 cm^{-1} suggests the presence of the Si-OH group, which disappears after calcination.



(A)



(B)

Fig 2. (b) FTIR spectral analysis of synthesized silica nanoparticles A) interpreted data B) FTIR analysis SHAMDUA**.**

XRD analysis:

The X-ray diffraction analyzes the physical nature of the particle as crystalline or amorphous. The XRD studies reflect a broad semi-crystalline peak was observed for SiO_2 nanoparticles, with a wide band at 2Θ of 22^0 (figure 2(c)). According to Bragg's law, the size of the nanoparticle can be obtained by the**,**

$D=K\lambda/B\cos\Theta_B$ Average size = 22.8899 nm D= 2.275 nm

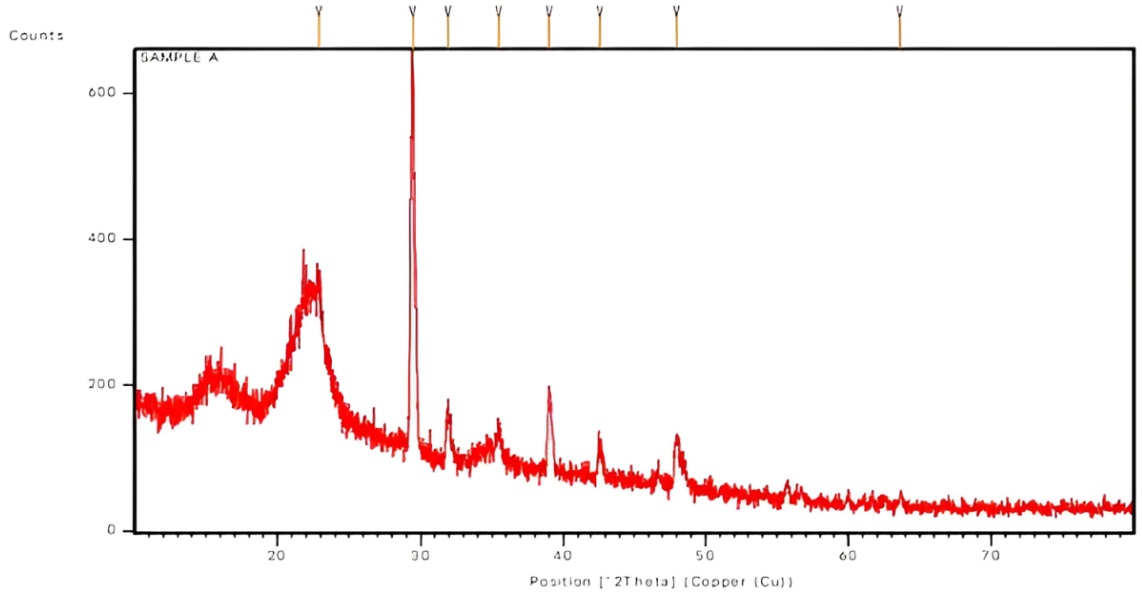
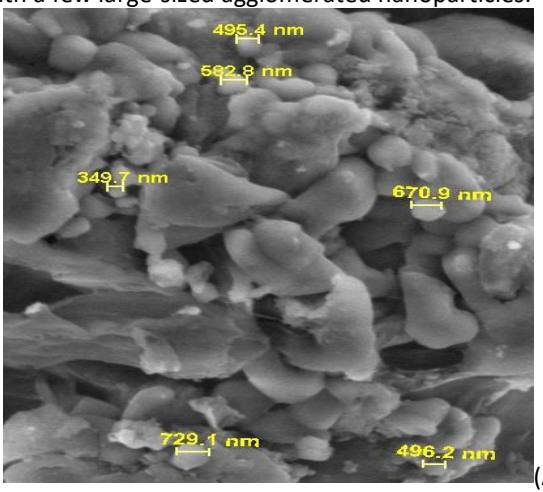


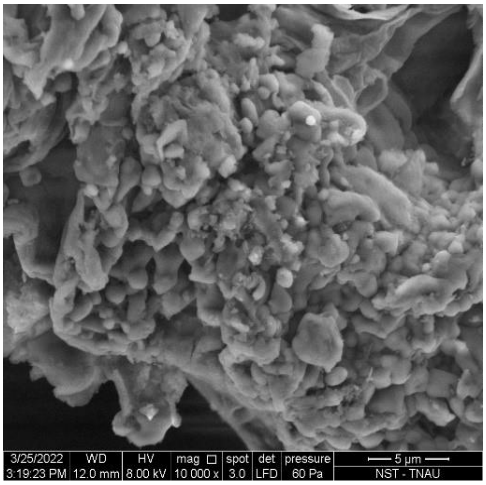
Fig 2. (c) The XRD patterns of the synthesized silica nanoparticles which have an average size approximately 22nm.

SEM analysis:

Scanning electron microscopy (SEM) analysis was done to determine the size and morphology of the silicon nanoparticle synthesized from the bamboo leaves. Figure 2(d) depicts the silicon nanoparticle possesses a spherical structure and an average size of 349.7 nm. It was visible in the image that the SiO_2 nanoparticles were observed with a few large-sized agglomerated nanoparticles.



(A)



(B)

Fig 2(d): SEM analysis of synthesized silica nanoparticles A) the size of the silicon dioxide nanoparticles B) surface analysis of the silicon nanoparticles.

Isolation of PSB:

Ten rhizobacteria were identified from the rhizospheric region of the soil of *Tectona grandis* using enrichment media. Among these, two strains were found to produce a halo zone around their colonies. These two isolates were labeled as PSB1 and PSB2, indicating their phosphate solubilizing activity. To evaluate their potential in converting inorganic forms of phosphorous into the solubilized form, the halo zone of phosphate solubilization was observed on Pikovaskaya's agar supplemented with 2% TCP. The results showed that PSB1 exhibited the highest solubilization efficiency and solubilization index, surpassing PSB2 with a value greater than 1.25 (Fig 3(b)).

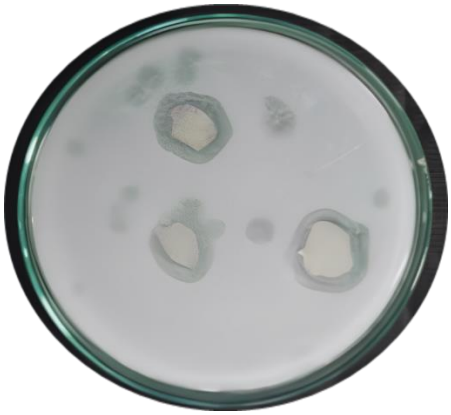


Fig. 3 (a) Phosphate solubilizing activity of isolate (PSB 1) from *Tectona grandis* rhizopsheric soil on Pikovaskaya's agar supplemented with 2% of TCP. The PSB1 which produces a solubilization efficiency of 80% (SE) and the solubilizing index of 2.24 (SI).

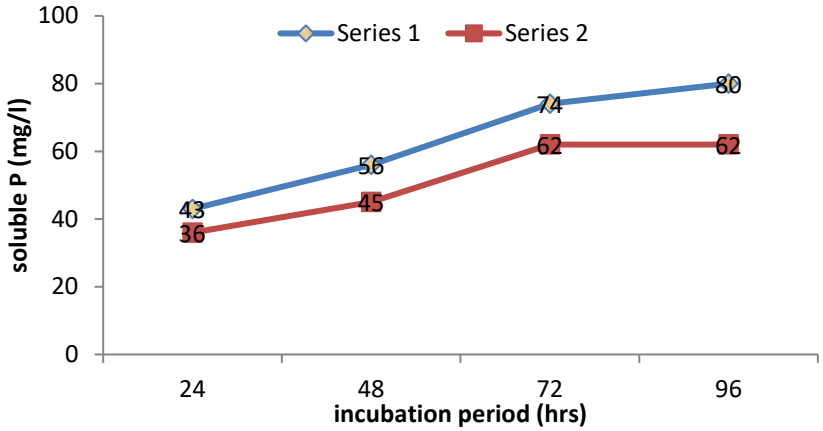


Fig. 3 (b) Aluminium phosphate solubilization profile of PSB1 (series 1) and PSB 2 (series 2) isolates as a function of incubation period.

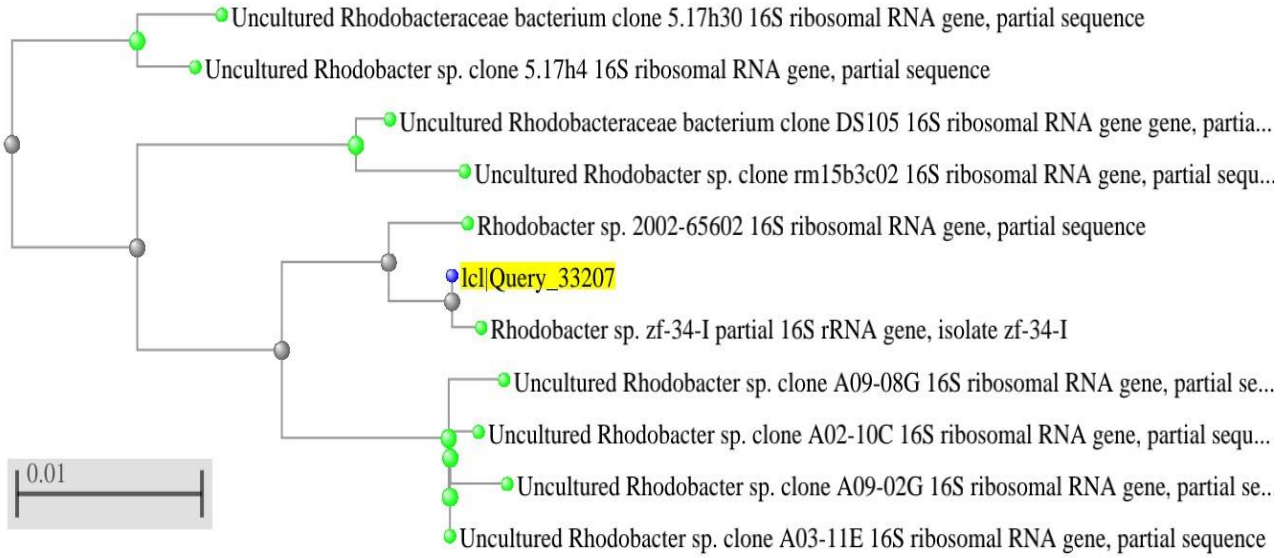


Fig. 3. (c) Phylogenetic tree of *Rhodobacter* species (PSB) which isolated with GenbankID: OR56709.1 (Karthikeyan *et al*)

Quantitative determination of Indole Acetic acid:

The selected PSB 1 was tested for IAA and quantified at 540 nm by supplementing the growth media with L-tryptophan. PSB 1 (15 µg/ml) displayed the highest production of IAA. The PSB1 isolate produced a pink color after half an hour of dark incubation of adding Salkowskis reagent.

Production of Organic acid, Siderophore, Ammonia, and HCN:

The organic acid production was observed for the isolate PSB 1 by changing the color of the media. Another indirect mechanism, Ammonia (NH_3) production, was checked for the isolate. Isolate showed positive for ammonia production. The HCN production was observed for the isolate PSB1, which turns the filter paper from reddish brown to orange in color. In addition to this, the isolate PSB1 exhibited a color change of greenish blue CAS agar media to yellow indicating significant production of Siderophore. The ACC deaminase activity of the isolate PSB1 showed positive in the Dworkin Foster Salts medium (Table 2(b)).

Table 2. Cultural characteristics of the *Rhodobacter sp.*

Strain	24	48
PSB1		
0.1	1.213	1.970
0.5	1.900	2.047
1	2.042	2.540
5	2.262	3.001
10	2.286	2.346
15	2.498	3.002

Table 2. (a): The quantitative measurement of IAA activity of PSB at 540 nm of the selected isolate (*Rhodobacter sp.*)

ISO	Media	Shape	Size of colony(cm)	Color	Elevation	Margin	Opacity	Gram staining	Motility
A	+	Oval	0.53	White	Raised	Entire	Transparent	G $^{-ve}$	+

Table 2. (b): The Plant growth promoting rhizobacterium activity of the *Rhodobacter sp.* in in-vitro conditions.

Iso	PSB	IAA	ACC	Fe	HCN	Organic acid	NH_3	Nitrogen	Antagonistic
R1	80%	+++	+++	+	+++	+++	+++	+++	++

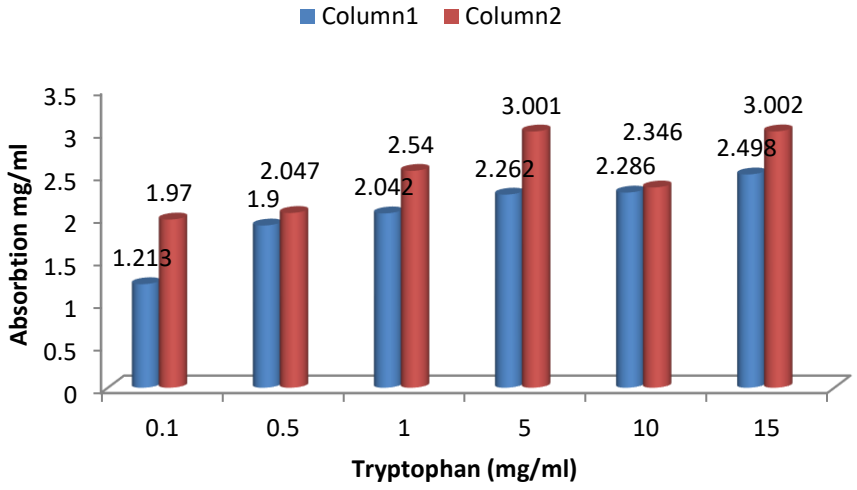


Fig 3. (d) IAA production profile of PSB1 as a function of incubation period and of L- tryptophan concentration in which column 1 indicates 24 hrs. and column 2 indicates the 48 hrs.

Synergistic Activity:

The research focused on evaluating the efficacy of a combination of phosphate-solubilizing bacteria and synthesized nanoparticles. The qualitative test showed the absence of a halo zone around the nanoparticle disc where the organism was swabbed on the plate. Through quantitative analysis using the Time Kill Kinetic assay, it was observed that the media containing nanoparticles had a higher cell count (335 $\times 10^{-1}$) compared to those without nanoparticles (323×10^{-1}) as shown in Fig 3(e). This suggests a direct correlation between the presence of nanoparticles and cell growth, indicating that nanoparticles contribute significantly to enhancing cell proliferation. Therefore, the synergy between microorganisms and nanoparticles presents a promising avenue for the development of an improved Nano fertilizer.

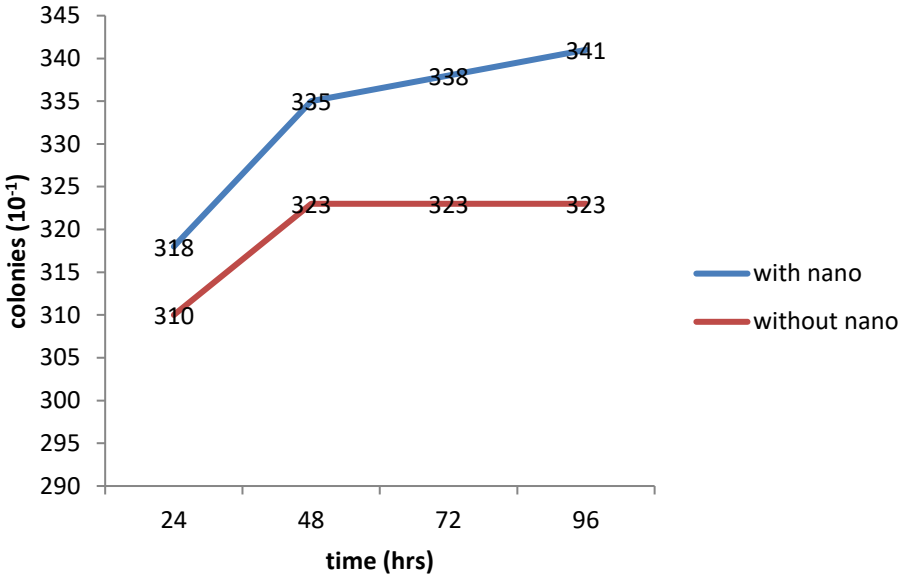


Fig 3. (e) Quantitative analysis for synergistic activity for the combination of both Nanoparticle and the *Rhodobacter sp.*

Pot trial analysis:

The combined treatment of nanosilicon dioxide and *Rhodobacter sp.* results in better growth and improvement in the *Zea mays*. Tables 3(a), 3(b), and 3(c) show the significant difference between the plant's phenotypic characters and the biochemical characteristics of the plants. Table 3(d) and (e) describe the soil enzyme analysis and its difference in the treatments.

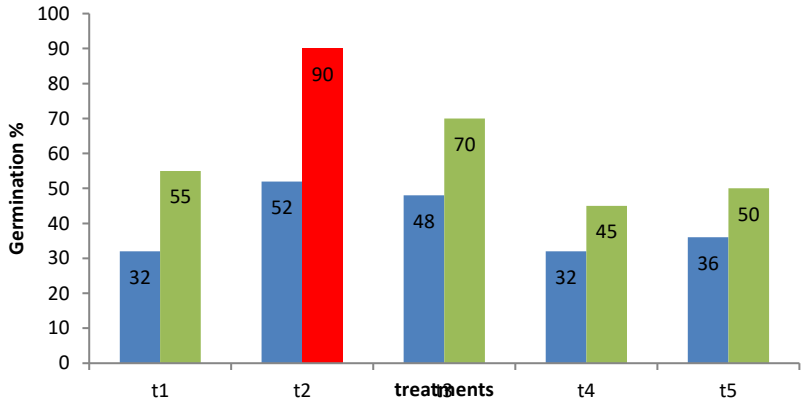


Fig 4. The germination percentage of the seeds in the 15th and 30th day of sowing seeds. **T2** (SiO_2+ PSB) shows high germination percentage.

Table 3. (a) The table represents the height, girth and number of leaves in the 15th day and 30th day of different treatments. a, b, c, d represents the treatments are significantly different from each other.

Treatment	Height -15(cm)	Height -30(cm)	Girth -15(cm)	Girth -30(cm)	Number of leaves-15	Number of leaves -30
T1	22.06±2.7^{a}	51.90±1.6^{b}	0.23±0.02^{ab}	0.35±0.05^{bc}	2.6±0.57^{a}	5.6±0.57^{c}
T2	39.10±1.8^{b}	46.41±4.0^{b}	0.33±0.07^{b}	0.43±0.07^{c}	3.3±0.57^{a}	7.3±0.56^{d}
T3	26.70±12.8^{ab}	27.32±12.4^{a}	0.20±0.10^{ab}	0.25±0.10^{ab}	2.6±0.57^{a}	2.6±0.57^{a}
T4	21.16±9.24^{a}	31.26±1.16^{a}	0.18±0.03^{a}	0.20±0.03^{a}	2.3±0.57^{a}	3.6±0.57^{b}
T5	24.00±7.2^{a}	28.42±7.7^{a}	0.23±0.05^{ab}	0.11±0.90^{a}	2.8±0.56^{a}	4.0±0.00^{b}

Values within a column followed by single letters (a,b,c,d) show significant varietal difference by Duncan's test.

Table 3(b). Total chlorophyll and carotenoid of maize plants under treatments after 30th day of sowing.

Treatment	Chlorophyll (663 nm) mg/g	Chlorophyll (645 nm)mg/g	Carotenoid(480 nm)mg/g
T1	0.316±0.33^{a}	0.253±0.26^{a}	1.39±0.82^{b}
T2	0.818±0.27^{b}	0.629±0.25^{b}	1.04±1.03^{ab}
T3	0.185±0.22^{a}	0.208±0.18^{a}	0.43±0.11^{ab}
T4	0.183±0.66^{a}	0.218±0.04^{a}	0.52±0.08^{ab}
T5	0.600±0.05^{a}	0.048±0.03^{a}	0.193±0.09^{a}

Values within a column followed by single letters (a, b) show significant varietal difference by Duncan's test.

Table 3(c). Total sugar content and protein of maize plants under different treatments.

Treatment	Total sugar content (620 nm) mg/g	Protein (595 nm) mg/g
T1	0.53±0.01^{a}	0.053±0.01^{a}
T2	0.09±0.02^{b}	0.097±0.02^{b}
T3	0.05±0.02^{a}	0.052±0.02^{a}
T4	0.04±0.01^{a}	0.031±0.01^{a}
T5	0.03±0.03^{a}	0.022±0.02^{a}

Values within a column followed by single letters (a, b) show significant varietal difference by Duncan's test.

Table 3(d): Alkaline phosphatase and ƀ glycosidase activity in the soils of maize plants under different treatments.

Treatment	Alkaline phosphatase (400 nm) µgmol/min/mgprotein	ƀ Glucosidase (410 nm)µgmol/min/mgprotein
T1	0.617±0.05^{c}	0.140±0.015^{b}
T2	0.768±0.01^{d}	0.442±0.026^{d}
T3	0.329±0.01^{b}	0.391±0.033^{c}
T4	0.627±0.08^{c}	0.670±0.022^{a}
T5	0.228±0.015^{a}	0.290±0.012^{a}

Values within a column followed by single letters (a, b, c, d) show significant varietal difference by Duncan's test.

Table 3(e): Invertase and Dehydrogenase activity in the soils of maize plants under different treatments.

Treatment	Invertase(508nm)µgmol/min/mgprotein	Dehydrogenase (485 nm)µgmol/min/mgprotein
T1	0.26±0.02^{b}	0.237±0.07^{a}
T2	0.88±0.09^{c}	1.375±0.33^{b}
T3	0.86±0.17^{c}	0.363±0.08^{a}
T4	0.16±0.06^{ab}	0.252±0.07^{a}
T5	0.03±0.46^{a}	0.247±0.02^{a}

Values within a column followed by single letters (a, b, c) show significant varietal difference by Duncan's test.

DISCUSSION

In all treated seeds, the percentage of maize seeds that germinated was higher than in the control. Additionally, earlier studies have shown that using nanomaterials at lower concentrations can improve the growth of several crop species as well as seed germination. (Hernandez-Viezcasa *et al.* 2011; Prasad *et al.* 2012; Mousavi-Kouhi *et al.* 2015). The diverse functions that silicon plays in plants, especially in reducing different kinds of stress, have

recently received a lot of attention. Rastogi *et al.* (2019) documented evidence demonstrating that silicon is important for improving plant resistance to a variety of stresses, such as heat stress, water stress, salt stress, heavy metal toxicity, and enhanced seed germination.

The combination of nSiO2-PSB-treated plants showed an increase in plant growth. According to Karthikeyan *et al.* (2008), beneficial microorganisms have the capacity to improve the effectiveness of the shoot and root systems by providing seedlings with critical amounts of nitrogen and phosphorous, which are necessary for growth. Gibberlic acid (GA) levels may have increased, which could explain the observed rise in plant height because GA mainly controls shoot elongation. Santana *et al.* (2016) found that PSBs improve the availability of other trace elements, like siderophores, which in turn promotes plant growth in an indirect manner. Plant development is promoted by PSBs through the production of phytohormones such as auxins, gibberlins, cytokinins, or polyamides, according to Mittal *et al.* (2008), Vikram *et al.* (2008). Yousefi *et al.* (2011), and Santana *et al.* (2016). When SiO2 and PGPRs were used to treat maize plants, Kukreti *et al.* (2020) found that the plants' physical and biochemical characteristics increased. The current study implies that the combination of nSiO2 and *Rhodobacter* sp. increased the plant height. The girth of the maize plant was directly proportional to the plant height. Sahandi *et al.* (2011) suggested that the inhibition of ethylene action reduces abscission, which can lead to an increase in the number of leaves. The increase in the production of ACC helps to inhibit ethylene. In the present study, PSB-produced ACC, in combination with nano silica, reduced ethylene and increased the number of leaves. Terry *et al.* (1983) observed that chlorophyll content is the index of the total light-harvesting complex and electron transport component present in chloroplast membranes. We report a significant ($p < 0.05$) increase in chlorophyll and carotenoid content in treated maize plants compared to that of the control. The maximum amount was observed in the combined treatment of nSiO2 and *Rhodobacter sp.* Zhang *et al.* (2019) reported that the utilization of silicon dioxide led to enhancements in carotenoid content, antioxidant enzyme activity, stomatal conductance, and water use efficiency in tomatoes. According to Rastogi *et al.* (2017, 2019), Si-NPs will directly interact with the plants to improve their growth and yield due to their size and physiological parameters. The treatment of single PSB (50%) was less when compared to the individual treatment.

We reported an increase in sugar content and protein in the combined treatment rather than control. Arora *et al.* 2012 observed that the use of gold nanoparticles at 25 and 10 ppm in *Brassica juncea* results in the enhancement of the sugar and chlorophyll contents of the plant. Zn oxide nanoparticles also have a great positive impact on the total leaf protein, increasing by 27% after application of the nanoparticle (Raliya and Tarafdar, 2013). Soil is an important factor involved in plant growth (Lal. 2015). In the present study, the combination of the treatment, i.e., the bacterial culture Rhodobacter sp. and the nanocompound (nSiO2 + PSB), showed the maximum level of invertase and dehydrogenase enzyme activity in the soil. Subhani *et al.* (2001) studied the dehydrogenation and hydrogenation of the dehydrogenase enzyme and its ability to be involved in all the enzymatic reactions of the living system. Thus, from this, we can say that the combined treatment doesn't have any negative impact on the soil environment. Dehydrogenases are considered as the index in the microbial activity of the soil (Moeskops *et al.* 2010), and the lack of the enzyme is an indicator of pollution (Kaczyńska *et al.* 2015). We reported an increase in the amount of dehydrogenase in treatment 2 more than the other treatments. The present study results show that the addition of nSiO2 with the bacterial culture enhanced the activity of the bacteria more than when it was applied individually. Lynette and Sunil (2017) observed that invertases are glycoside hydrolases and occur mostly in all microbes. We reported that the activity of the invertase enzyme increased in the combined treatment compared to the application of individual treatments of both the nanoparticle and the bacterial culture.

Izabela *et al.* (2014) suggested that the use of nCuO negatively affected the enzymatic activities of the microbes in the soil. We observed that all the treatments were significantly different from the control. The individual treatments of nSiO2 and PSB increased the activities of beta-glucosidase and invertase enzymes. However, the chemical fertilizer did not show any increase in enzyme activity, except for alkaline phosphatase, which showed a slight increase. Bharti *et al.* (2020) stated that soil enzymes play a crucial role in recycling various nutrients, and nanoparticles can act as effective chelators for these nutrients. Patel (2023) reviewed an alternative method currently being investigated, which involves integrating biofertilizers with nanoparticles to develop nano biofertilizers. The results suggest that the application of nano silicon dioxide and the phosphate solubilizing bacteria *Rhodobacter sp*. as a nanocomposite can enhance plant growth and soil activity through the enhancement of phenotypic and biochemical characteristics.

CONCLUSION

This study concludes that the combination of nanosilicon dioxide and phosphate-solubilizing bacteria *Rhodobacter sp*. increased plant growth. The nanoparticle from biowaste is an economically cheaper method, and the *Rhodobacter sp*. contains ascorbic acid, which increases the photosynthetic activity of plants. Overall, it has a positive impact on the soil as well as the plant. Nowadays, tons of inorganic fertilizers are produced, and they act antagonistically on the environment. As an alternative to this, we can use the nanocomposite without harming the soil's health.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest or personal relationships that could have appeared to influence the work reported in this paper.

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