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Regulatory Role of Nanoporous Silica on Dicot *Cicer arietinum* and Monocot *Sorghum bicolor*

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ABSTRACT

In the present era of rising population and pollution, the demand of commercialized nano formulations in agricultural fields is increasing. Nanoporous silica has emerged as a novel tool for drug delivery and diagnosis. However, very few studies have been conducted to elucidate its effects on plants and animals. It is essential to study the effects of nanoporous silica on consumable plants because they are being absorbed by these primary producers and successively passed along the food chain. Also, food and immunity are two sides of a coin. Gut flora assists in immunity. This age belongs to a diet that has the ability to enhance immunity. Therefore, fertilizers that eventually enter the intestine should be beneficial to the bacteria residing in it. This experiment aims at studying the morphological characters of nanoporous silica, investigating its effects on various biochemical pathways of model crop plants like dicotyledonous *Cicer arietinum* and monocotyledonous *Sorghum bicolor* and on the growth and viability of dominant gut flora *Bacillus coagulans*. The results of this study establish that it can positively regulate certain biochemical pathways in a size dependent and dose dependent manner in plants. It has been found to increase growth of *B. coagulans* over specific time scale.

***Key words*:** Nanoporous silica, Gut flora, Mesoporous, Osmotic stress, Oxidative stress

In the growing era of nanoscience and nanotechnology, advancement in the search for novel nanoparticles has led to synthesis or isolation of nanoparticles with unique chemical structure and properties. These nanoparticles either exist in Nature or are manufactured in industries. In the past few years, one such particle, with nanoporous structure, namely mesoporous silica nanoparticles (MSNs) or nanoporous silica has gained importance owing to its eccentric properties such as solid framework, large surface area, porous structure, active surface with honeycomb-like structure high loading capacity, low toxicity, higher biocompatibility, and more stability [1]. Silica is the second most abundant element on Earth's crust due to the age-old weathering of rocks, sedimentation and biosilicification process in both terrestrial and aquatic organisms. Nanoporous silica also exists in Nature the synthesis of which depends on the biosilicification in organisms such as diatoms, sponges, etc. [1]. The coastal areas have huge deposition of nanoporous silica as sea water recedes leaving behind the diatom skeletons as diatomaceous earth. Besides the structural novelty of MSNs, they have surface charge at both outer and inner surfaces [2]. It has been found that drug loading in mesoporous nanoparticles and liquid transport rates through mesoporous membranes are directly related with internal surface charges [3]. It has also been seen that with a decrease in salt concentration or a decrease in pore size, the bulk electric potential became different than zero [2]. Moreover, the permeability of solvents through an artificially synthesized MSN, namely MCM 48, reduces from water to propanol indicating that the various types of interactions of the solvent molecules with surface of MCM 48 such as hydrophobic–hydrophilic interactions between the pore walls and the solvents, and/or to alkoxylation of surface ≡Si–OH groups by ethanol and 1-propanol affect the flow of solvents through the pores [3]. Thus, how a mesoporous silica behaves in a solution is an interesting topic in nanotechnology.

Presently, nanoporous silica is being extensively used as effective delivery vehicles for a variety of biocides to fight against various diseases including bone/tendon tissue engineering [4-7], diabetes [8-9],, inflammation [10], AIDS [11] and cancer [12]. It has also found application in optics, photonics, sensing, biosensing, filtration, microfabrications, protein separation, catalyses, drug delivery, etc. [1]. With the

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growing demand of commercial nanotechnology products, and continuous large scale biosilicification and deposition of nanoporous silica, human exposure to MSNs is increasing with every passing day. There might be a size dependent hazardous effect of nanoporous silica on human health owing to it enhance ability to penetrate intracellular targets in the lung and systemic circulation. Biocompatibility is essential for the development of industrial nanoparticles [13].

Till date, Silica has been proved to be useful in plant metabolism. Plants deprived of Si are often weaker structurally and more prone to abnormalities of growth, development and reproduction and it is the only nutrient which is not detrimental when collected in excess [14]. It has also been already found that metal toxicity, salinity, drought and temperature stresses can be alleviated by Si application [15-17]. However, not much study is done on the effects of nanoporous silica on plants.

The nanoporous silica that is either discarded in the environment as industrial wastes or is deposited as a result of biosilicification is taken up by plants along with other inorganic materials and passed on to humans and other animals. Therefore, studying its effects on crop plants may provide deeper insights into its role in plant growth and development and toxicological effects, if any. Moreover, studies on effects of high doses of amorphous silica on human health showed that it may result in acute pulmonary inflammatory responses, which could induce long-term effects [18]. Nanoporous silica of <300nm size showed no apparent cytotoxicity in different human cell lines but those with larger size and larger pores caused concentration- and time dependent inhibition of cellular respiration and found to be toxic to the isolated mitochondria in HL-60 cell lines [19]. But a prominent way by which mesoporous silica might enter the human body is through the gut. Moreover, the bacteria residing within the human gut not only assists in digestion but also produces huge amount of antibody [20]. Thus, studying the effects of nanoparticles on human gut flora is becoming essential in the present day where the need of highly developed immune system is increasing with introduction of newer disease-causing entities such as COVID-19. A weakened immune system as a result of consumption of environmental pollutants might prove to be fatal in future.

In this study, we have focused into the structural characteristics of nanoporous silica obtained from Gujarat, India and its effect on morphology, photosynthetic pigments, biochemistry and oxidative stress of a dicot model *Cicer arietinum* (gram) and a monocot model *Sorghum bicolor*. We have also done a brief study on the effect of nanoporous silica on the growth of *Bacillus coagulans*, an essential, dominant human gut bacterium [21] to study the consequence of consumption of nanoporous silica as an element of food chain/web.

MATERIALS AND METHODS

Collection of Nanoporous silica and seeds

Nanoporous silica was obtained from Gujarat, India. The organically grown Bengal gram (*Cicer arietinum*) and *Sorghum bicolor* were collected from Rajasthan.

Filtration: For size separation of the nanoporous silica (MSNs), membranes filters of pore size 0.22 µm and 1.2 µm were used (Merck Millipore, USA). Briefly a solution of nanoporous silica was filtered and the filtrate was collected and stored for size characterization.

Characterization of the particles (DLS, TEM, SEM): Physicochemical characterization of nanoporous silica was carried out using dynamic light scattering (DLS) (MALVERN Zetasizer, UK), transmission electron microscope (TEM) (JEM-2100F, JEOL Ltd., Japan), scanning electron microscope (SEM) (FEI Quanta 250 FEG-SEM< Thermo Fisher Scientific, USA), and, atomic force microscopy (AFM) (Park XE 70, USA).

Seed sterilization and plantation: Seeds were soaked in water for 2 hours. Thereafter, surface sterilization was performed using 5% Sodium hypochlorite (Merck Millipore, USA) for 20 minutes, followed by rinsing with deionized water. They were kept overnight in dark at room temperature for germination. In pots, 10 g perlite was taken and to it 1 ml, 2 ml and 5 ml of nanoporous silica (Mesoporous silica nanoparticles, MSNs) with <200 nm diameter and 300-600 nm diameters were added respectively (deionized water served as control). Naming was done to distinguish the seedlings from each other (Gram: GC for control, GS1 for 1ml of <200 nm MSN, GS2 for 2 ml of <200 nm MSN, GS3 for 5 ml of <200 nm MSN, GS4 for 1 ml of 300-600 nm MSN, GS5 for 2 ml of 300-600 nm MSN, GS6 for 5 ml of 300-600 nm MSN, GSM for crude MSN; *Sorghum*: SC for control, SS1 for 1 ml of <200 nm MSN, SS2 for 2 ml of <200 nm MSN, SS3 for 5 ml of <200 nm MSN, SS4 for 1 ml of 300-600 nm MSN, SS5 for 2 ml of 300-600 nm MSN, SS6 for 5 ml of 300-600 nm MSN, SSM for crude MSN). Germinated seeds were planted and left for seven days at 8 hours day:16 hours night growth condition at 25°C.

Morphology: After seven days, seedlings were taken out, washed with double distilled water and growth parameters in terms of root length, shoot length, rootlet numbers were recorded. For dry weight and moisture percentage they were oven dried at 80°C for 24 hours.

Photosynthetic pigments

Chlorophyll a, b and carotenoids and xanthophyll were measured using the protocol described by Sumanta *et al.* [22]. Briefly, accurately weighted 0.5g of fresh plant leaf sample was taken, and homogenized in tissue homogenizer with 10 ml of 95% ethanol. Homogenized sample mixture was centrifuge for 10,000 rpm (Eppendorf 5804 R, Germany) for 15 min at 4°C. The supernatant were separated and 0.5 ml of it was mixed with 4.5 ml of the solvent. The solution mixture was analyzed for Chlorophyll-a, Chlorophyll-b and carotenoids content in UV-spectrophotometer (UV-1800, Shimadzu, Japan). The equations used are as following (concentration in µg/ml):

$$\text{Chlorophyll-a} = 13.36\ A_{664} - 5.19\ A_{649}$$

$$\text{Chlorophyll-b} = 27.43\ A_{649} - 8.12\ A_{664}$$

$$\text{Carotenoids + Xanthophyll} = (1000\ A_{470} - 2.13\ C_a - 97.63\ C_b)/209$$

FITC labeling of nanoporous silica and uptake by seedlings: Nanoporous silica (MSNs) was labeled with FITC (Sigma, USA) according to the method described by Guo *et al.* [23]. Briefly, nanoporous silica and FITC were mixed to give the final concentration of 2.5 mg/ml nanoporous silica and 0.1 mg/ml FITC and left overnight. Thereafter, FITC was quantified, using a UV-Vis spectrophotometer, according to its absorbance peak at 488 nm. Finally, seedlings were

immersed in the solution after successful loading of the dye onto the silica pores for 8 hours, roots and shoots were dissected and observed under the fluorescence microscope (Nikon, Eclipse H600L, Japan) under green filter.

Antioxidant assay

Enzyme assay: For enzymatic assay, root and shoot extracts were prepared homogenizing the 5g plant tissues in 5ml lysis buffer (50mM Tris–HCl, pH 7.5, 1mM EDTA, 150 mM NaCl, 1% NP-40, 1 mM DTT, protease inhibitor cocktail) (Sigma, USA), incubating for 30 min on ice followed by sonication (CITIZEN DIGITAL ULTRASONIC CLEANER, CD4820, India). Thereafter, the solutions were centrifuged at 11000 rpm for 10 minutes and the supernatant was used for the following assays [24].

Catalase: The activity of catalase (CAT) was measured according to the procedure described by Aebi et al. [25] with slight modifications. Briefly, reaction mixture was set up by 50 mM sodium phosphate buffer, 10 mM H_2O_2, 0.1 ml enzyme extract and 0.4ml deionized water to give a final volume of 3 ml. The H_2O_2 was added in the end to start the reaction. The decrease in absorbance was recorded for 1 minute at 15 seconds interval at 240 nm using spectrophotometer.

Ascorbate peroxidase: The activity of Ascorbate Peroxidase (APX) was measured by the process described by Zhang et al. [26]. Briefly, a 1 ml reaction mixture containing 50 mM potassium phosphate buffer (pH 7), 10 µl enzyme extracts, and 0.5 mM ascorbate was set up and to it 0.1 mM H_2O_2 was added to initiate the reaction. The decrease in absorbance of the oxidized ascorbate at 290 nm was recorded using spectrophotometer.

Total superoxide anion (O^{2-}): Total O^{2-} was measured using the above extract and with modifications of the procedure described by Doke [27]. The O^{2-} content was determined based on its ability to reduce NBT. Fresh leaf tissues (0.5 g) were excised and immersed in 10 mM potassium phosphate buffer (pH 7.8), containing 0.05% nitro blue tetrazolium and 10 mM sodium azide. The sample was incubated for 1 hour at room temperature. Following incubation, 2 ml of this reaction solution was heated at 85°C in a water bath for 15 min and cooled in an ice bath. Optical density of solution was determined at 560 nm for 15 min using a spectrophotometer. The O^{2-} content was expressed as the increase in absorbance per unit dry weight.

Osmotic stress analyses

Total proline: Fresh roots and shoots were taken and diluted 20 to 50 times (w/v), typically in a 70:30 ethanol:water mixture (v/v). Reaction mixture was set up using 1% ninhydrin (w/v) in acetic acid 60% (v/v), ethanol 20% (v/v) and kept away from light. To 100 µl reaction mixture, 50 µl ethanolic extract was added. In case of standard curve, 50 µl of 1-0.4-0.2-0.1-0.04 mM proline standard prepared in 70:30 ethanol:water (v/v) was added to the reaction mixture. The mixtures were heated at 95 ^{0}C (either in block heater or water bath) for 20 min. After cooling at room temperature, they were centrifuged at 2500 rpm for 1 minute. To microplated well, 100 µl of the mixture was transferred and read at 520 nm in ELISA reader (Multiscan FC, Thermo Fisher scientific, USA) [28].

Total free amino acids: Total free amino acids was estimated by ninhydrin method as proposed by Moore and Stein [29]. Briefly, 500 mg plant tissue was homogenized with 80% ethanol, centrifuged and supernatant was used for the assay. An aliquot (0.1 ml) of the ethanolic extract was mixed with 1 ml ninhydrin reagent and boiled for 20 minutes. To it 5 ml diluent (1:1 propanol and water) was added and the absorbance was measured at 570 nm against reagent blank (0.1 ml of 80% ethanol, 1 ml ninhydrin, 2 ml water and diluent). Alanine was used as standard.

Total protein, total carbohydrate and total lipid: Total protein, lipids and carbohydrate assays were performed simultaneously from a single extract using the method suggested by Chen *et al*. [30] with modifications. In short, extracts were prepared by homogenizing 10-50 mg plant tissue in 1.5 ml R1 (25% methanol in 1N NaOH), and subsequently centrifuged at 10000 rpm for 10 minutes. Two aliquots of the lysate (0.2 ml) (one to act as blank and the other as sample) were taken to measure the total carbohydrates or the dissolved carbohydrates in the supernatant following centrifugation, using the anthrone method. The remaining sample was saponified by heating at 100°C for 30 min and cooled down to room temperature. Two aliquots (0.1 ml each, one to act as blank and the other as sample) were transferred to Eppendorf tubes and centrifuged; the supernatant was used for estimating proteins using the microbiuret method [31]. In this method, an aliquot (0.05 ml) of alkaline copper sulphate (0.21% $CuSO_4.5H_2O$ in 30% NaOH) was added to 0.1 ml of the samples and the absorbance was measured at 310 nm. This method was not affected by the presence of high concentrations of deoxyribonucleic acid (DNA). Bovine serum albumin (BSA) from Sigma–Aldrich was used as the standard for calibration. Another aliquot (0.5 ml) of sample was pipetted to an eppendorf tube containing 0.75 ml of a solvent mixture R2 (chloroform/methanol, 2:1, v/v) and vortexed for 2 min. The mixture was centrifuged at 12,000g for 2 min to get two phases. The top aqueous phase contained chlorophyll, while the lower organic phase contained total carotenoids and lipids. The absorbance of the lower phase was read at 260 nm (for lipids) after the organic phase was reacted with R3 (1 M triethanolamine:1 N acetic acid, 9:1, v/v) required for the lipid assay.

Preparation of Bacillus coagulans culture and treatment with MSN solution

This was performed according to the method described by Mukherjee *et al*. [21]. Briefly, the content of Vizylac capsule was dissolved in 0.85% sterile saline water. A loopful of suspension was streaked on agar plate containing Lactobacillus MRS Agar (HiMedia, India). The plate was incubated for 48 hours at 37°C. Now single isolated colony was inoculated in sterile MRS broth (HiMedia, India) at same temperature in 120 rpm for overnight. For the treatment, adjusted bacterial inoculums were incubated in test tubes in presence of range of MSNs to achieve the exposure concentrations of 200 µl, 400 µl and 1000 µl respectively. These amounts of MSN did not alter the pH of bacterial suspension (pH 6.5). Tube without nanoparticles was used as control. Tubes with bacteria and nanoparticles were mixed well by vortexing and incubated at 37°C in a dark shaker (120 rpm). The optical density was measured using spectrophotometer. The growth of bacterial strains was indexed by measuring optical density (OD) at 600 nm at various hours of intervals.

Statistical analyses: The experiment was performed using a randomized block design to minimize the differences due to the environment. For plant related experiments, thirty replicates for morphology, six replicates for moisture, thirty replicates for photosynthesis pigments and three replicates of other biochemical and enzymatic analyses for the *Cicer* and *Sorghum* were carried out to perform the Analysis of Variance (ANOVA). Whether each of the treatments was significantly different from control was analyzed. Statistical analysis was executed using SPSS software (IBM SPSS Statistics.). Significance of main effects was determined at the 0.05 probability level. The General Linear Model was used. The one-way ANOVA and Fisher's Protected LSD test was used to carry out the Post Hoc multiple pair wise comparison analysis to observe the mean difference. For bacteria related experiments, three replicates were used for each dose as well as control, ANOVA was used followed by Tukey's multiple comparison tests to evaluate significant differences between each group.

RESULTS AND DISCUSSION

Ccharacterization of the particles

Two separate batches of MSNs were extracted. As per the DLS data, the Z-average size of one was more than 200 nm and ≈395 nm (Fig 1A) and the other had Z-average size of ≈133 nm (Fig 1B) which is lower than 200 nm. Both the batches were used for plant growth (batch 1 was the <200 nm nanoparticles and batch 2 was the other one). The AFM data showed that the height of the particles ranged around 240 nm and the width was within 200 nm (Fig 1C). The TEM and SEM images showed the internal and external morphologies of the particles respectively (Fig 1D-E). The typical honeycomb like structure is clearly depicted in TEM image.

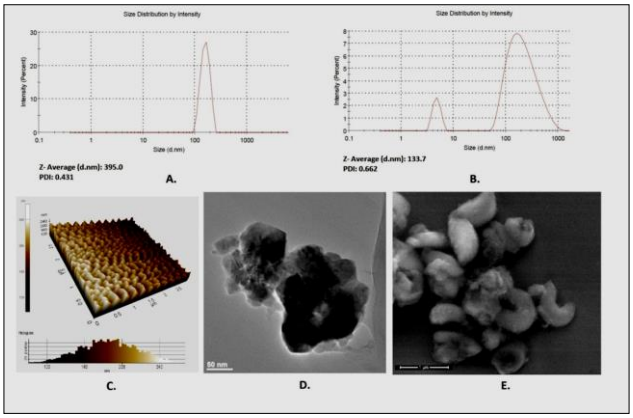

Fig 1 (A) DLS of 200-400 nm particles. (B) DLS of <200 nm particles. (C) AFM image. (D) FEG-TEM image. (E) FESEM image

Morphology of the seedlings: The seedlings were taken out after 8 days and studied in details (Fig 2).

Root length (cm), shoot length (cm) and rootlet numbers were recorded for each sample. According to the results, in case of *Cicer*, a trend is being followed in root length and shoot length, such that GS3>GS2>GS1>C with significant difference at 95% level. No significant changes were observed in GS4, GS5 and GS6 with respect to control. However, a huge difference was observed in crude Gujarat Silica samples, i.e., GSM, that being higher than C (Fig 3A). GS3 and GS2 had significantly higher number of rootlets than control C but no significant changes could be found in rest of the samples (Fig 3B). In case of *Sorghum*, root lengths of SS2 and SS3 was significantly higher than C (Fig 3A), shoot length and rootlet number was found to be significantly higher in SS3 and lower in SS4 with respect to control (Fig 3A-B). Thus, a marked effect of <200 nm particles could be observed in dicot *C. arietinum* at all concentration levels while only 2 ml and 5 ml of it was effective in monocot *S. bicolor*.

Fig 2 (A) Morphology of *Cicer* seedlings. (B) Morphology of *Sorghum* seedlings

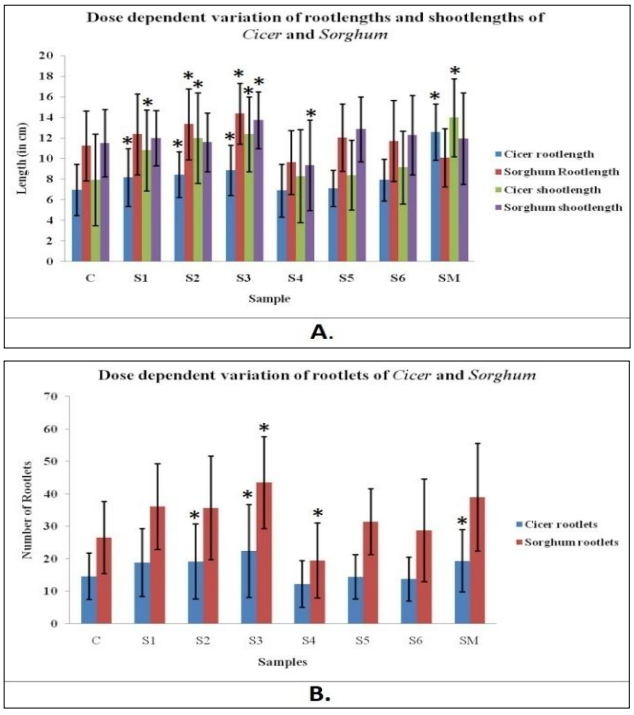

Fig 3 (A) Dose dependent and size dependent variation of root lengths and shoot lengths of *Cicer* and *Sorghum*
(B) Dose dependent and size dependent variation of root lengths and shoot lengths of *Cicer* and *Sorghum.* N=30. P value=0.05. *Means significant

Uptake of labelled MSNs by plant tissues

In UV-Vis spectroscopy, nascent MSNs do not give any peak at 480 nm wavelength while FITC labelled MSNs give a prominent peak at 480 nm. Uptake of the FITC labelled MSNs by *Cicer* and *Sorghum* seedling were studied under confocal microscope at 480 excitation. When root cross sections (transverse) were studied under the 480 excitation, very distinct green coloration was observed (Fig 4). This confirmed the uptake of FITC-MSNs by plant tissues.

Moisture content: No significant difference could be observed in morphology of treatments with respect to control in either of the two species. This proves that the MSNs have no significant effect on fresh weight, dry weight and moisture content of either of the plants. Hence, it is proved that nanoporous silica does not cause dehydration of the tissues.

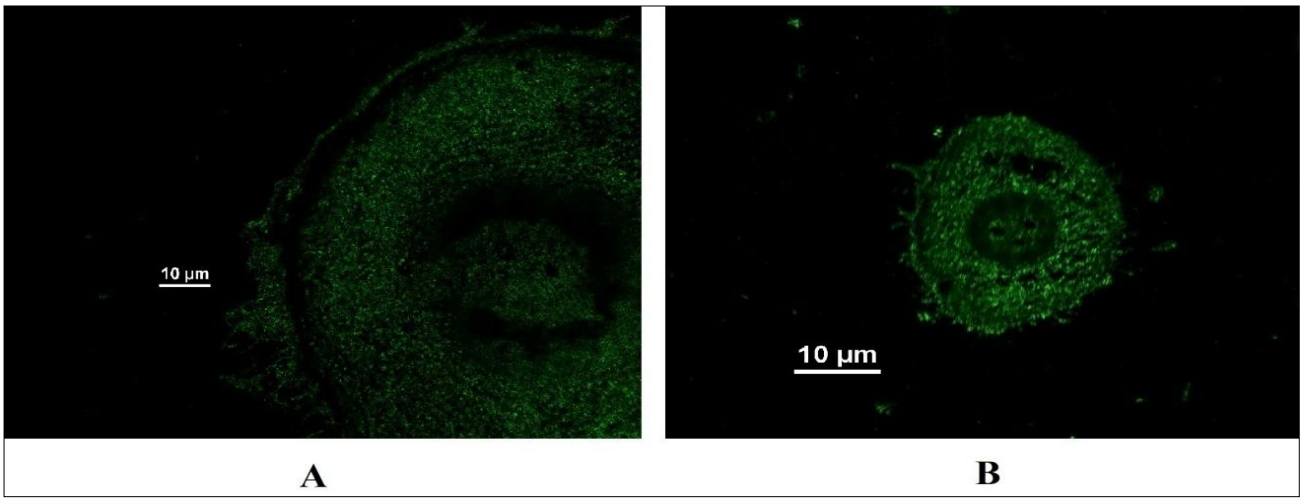

Fig 4 Uptake of FITC labeled mesoporous silica nanoparticles. (A) TS of *Cicer* root. (B) TS of *Sorghum* root

Photosynthetic pigments

In *Cicer* seedlings, significant changes in chlorophyll a from control only in GS3 and GS1 with the order GS2>C>GS3>GS1 in batch 1 MSNs, and in batch 2 all were significantly different from control with the order C>GS5>GS4>GS6. No significant change was observed in GSM. Chlorophyll b was significantly higher in GS2 in batch 1 MSNs, and in GS6 and GS4 with the order C>SS6>SS5>SS4 in Batch 2 MSNs. GSM had significantly higher amount of Chlorophyll b with respect to C. Carotenoid and xanthophyll did not change significantly (Fig 5A). In case of *Sorghum*, batch 1 showed significant changes in chlorophyll a from control with the order SS3>SS1>SS2>C, and in batch 2 only SS4 and SS6 were significantly lower than C. Chlorophyll b was significantly high in SS1 and SS3 in batch 1 MSNs with the order SS1>SS3>C, and lower in all batch 2 MSNs with the order C>SS5>SS6>SS4, and high in SSM. Carotenoid and xanthophyll were significantly high in SS3 with rest of batch 1 being insignificant. In batch 2, C>SS5>SS4>SS6. SSM was significantly lower than C (Fig 5B). Because of Chlorophyll-a's role in light harvesting complex and in PS-II reaction centre as electron provider to the photosynthetic electron transport chain, it is more susceptible to photodegradation than other photosynthetic pigments thereby being a more sensitive parameter to study the effects of external stimuli [32]. In *Cicer* seedlings, only GS2 had more Chlorophyll a than control, the rest of the samples having lower amount. Moreover, it had more Chlorophyll b, thereby proving that 2 ml of <200 nm MSNs was most effective dose in case of *Cicer* seedlings in increasing photosynthetic activity. However, crude nanoporous silica was not injurious to the seedlings in terms of photosynthetic activity. In *Sorghum*, SS1, SS2 and SS3 showed higher amount of Chlorophyll a, SS1 and SS3 showed higher amount of Chlorophyll b and only SS3 had more amount of Carotenoid and Xanthophyll as compared to Control, thereby proving that 5 ml of <200 nm MSNs significantly raised the photosynthetic pigments and was the most effective dose in terms of photosynthetic activation. The <200nm particles and crude MSN were beneficial to the seedlings, whereas the 300-600 nm particles somewhat reduced the photosynthetic activity of the seedlings.

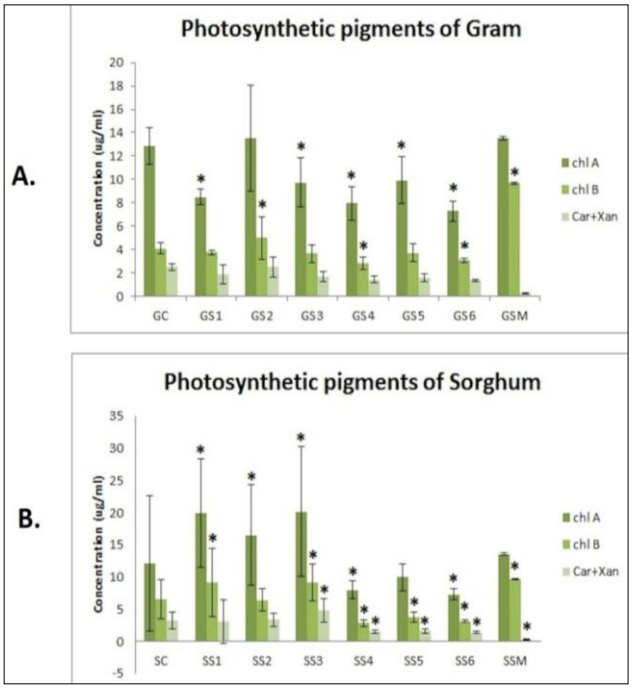

Fig 5 (A) Photosynthetic pigments of *Cicer*. (B) Photosynthetic pigments of *Sorghum*. N=30. P=0.05. *means significant

Total protein, lipid and carbohydrate: In *Cicer* seedlings, there were no significant difference in the root protein, shoot protein, root lipid, shoot lipid, root sucrose, shoot sucrose, root glucose and shoot glucose (Fig 6A-D). However, all roots of treatments had lower protein, higher lipids, higher or similar amount of sucrose and glucose and shoots had higher proteins, lipids and carbohydrates as

compared to that of control. This clearly states that in *Cicer* seedlings growing in all concentrations of MSNs, there is no detrimental effect of MSNs on the protein, lipid or carbohydrate.

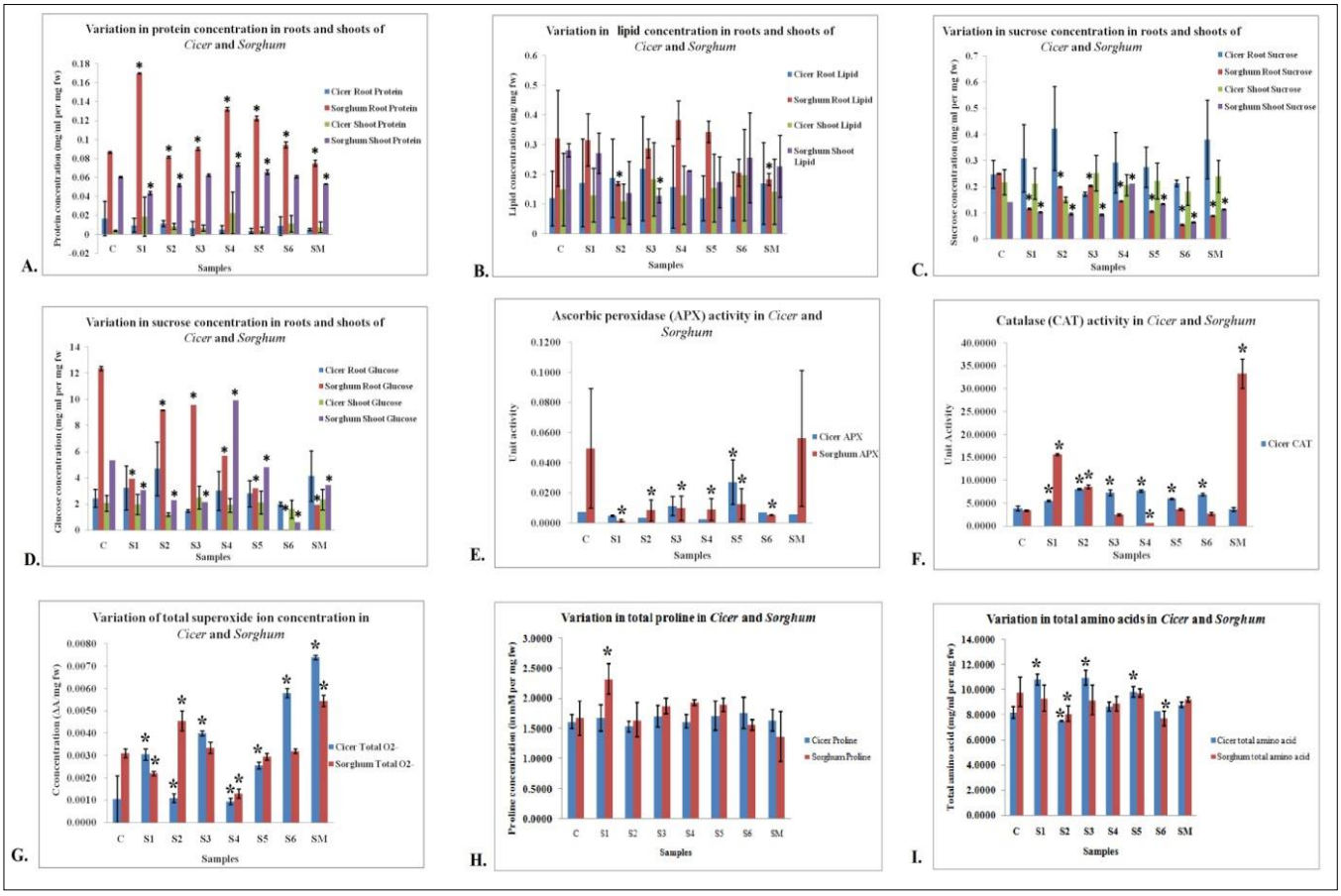

Fig 6 Variations in biochemical composition and antioxidant enzymes and total superoxide ion concentration in *Cicer* and *Sorghum* due to various doses of Nanoporous silica. (A) Variation in protein concentration (in mg/ml per mg fresh weight) in roots and shoots of *Cicer* and *Sorghum*. (B) Variation in lipid concentration (in mg per mg fresh weight) in roots and shoots of *Cicer* and *Sorghum*. (C) Variation in sucrose concentration (in mg/ml per mg fresh weight) in roots and shoots of *Cicer* and *Sorghum*. (D) Variation in glucose concentration (in mg/ml per mg fresh weight) in roots and shoots of *Cicer* and *Sorghum*. (E) Variation in Ascorbic peroxidase activity in *Cicer* and *Sorghum*. (F) Variation in Catalase activity in *Cicer* and *Sorghum*. (G) Variation in total superoxide ions concentration (ΔA/mg fresh weight) in *Cicer* and *Sorghum*. (H) Variation in total proline concentration (mM/mg fresh weight) in *Cicer* and *Sorghum.* (I) Variation in total amino acids concentration in terms of Ala (mg/ml per mg fresh weight) in *Cicer* and *Sorghum* N=3. P=0.05. *Significant

In case of *Sorghum* seedlings (Fig 6A), root protein showed significant changes in all the treatments with respect to control with the orders SS1>SS3>C>SS2, SS4>SS5>SS6>C and C>SSM. In shoot, total protein in SS2 and SS1 was significantly lower than C with the order C>SS2>SS1, that of SS4 and SS5 was significantly higher than C with the order SS4>SS5>SS6>C, and in SSM it was significantly lower than that of C. Thus, it can be said, that in seedlings growing in crude MSNs protein was being degraded in both roots and shoots, but in those growing in 1 ml 300-600 nm MSNs, protein is being synthesized rapidly in both root and shoot. Lipid content (Fig 6B) in roots of SS2 and SSM was found to be significantly lower than that of C roots and in shoots it was significantly lower in SS3. In rest of the cases here was no significant change in lipid control with respect to control. Lipid degradation is a sign of oxidative stress as ROS is known to cause lipid peroxidation and membrane damage [33]. Hence, it can be said that crude MSNs can induce lipid degradation and since both protein and lipid is lower in roots of SS2, so 2 ml of <200 nm MSNs might induce stress in roots of *Sorghum* seedling. Carbohydrate content (Fig 6C-D) was significantly different in all the treatments with respect to control in both root and shoot. In roots, sucrose of control was higher than all the treatments, such that for batch 1 it is directly proportional to doses (SS1 lowest) and for batch 2 it is inversely proportional to doses (SS6 lowest). Similar trend was found in glucose content of roots. Sucrose content and glucose content in shoot followed similar order. In first batch, C was higher and it decreased with increase in doses (SS1 highest), and in the second batch SS4 was higher than control with SS5 and SS6 being lower than control respectively (Fig 6). Soluble sugars are considered to be modulators of various processes associated with plant growth and development as well as stress responsive pathways [34]. Sucrose and glucose either serve as substrates of cellular respiration or as osmolytes maintaining cellular homeostasis [35]. Increased tolerance to environmental stress is caused by increase in soluble sugar levels. Thus, in SS1 and SS4 there is a chance of increased tolerance to stress.

Oxidative stress: Reactive oxygen species that are free radicals and non-radical molecules are components of various cell signalling pathways and acts as regulators of cellular responses in variety of physiological conditions and environmental changes [36]. Under normal physiological conditions, ROS are produced as a by-product of various cellular pathways and are, therefore, synthesized continuously at different cellular compartments such as chloroplasts,

peroxisomes, mitochondria, etc. [37]. On the other hand, ROS are also scavenged by an antioxidative defence system, whose components are often confined to certain cellular compartments [38]. In normal physiological conditions, there is equilibrium between ROS produced and ROS scavenged. An increase in ROS causes damage to proteins, DNA and lipids, while its decrease affects biological and physiological pathways [39]. As a consequence, there should be a balance between ascorbate peroxidase (APX), glutathione peroxidase (GPX), and catalase (CAT) activities. As reported by Apel and Hirt, if the balance of scavenging enzymes changes, compensatory mechanisms are induced (i.e., APX and GPX are up-regulated when CAT activity is reduced in plants). In *Cicer*, the unit activity of Catalase showed the trend of GS2>GS3>GS1>C and GS4>GS6>GS5>C with significance at 95% level (Fig 6F). However, Catalase activity in GSM was found to be the lowest of all. Unit activity of APX (Fig 6E), on the other hand showed negligible changes between C and GS1, GS2, GS3, GS4, GS6 and GSM, with only GS5 being significantly higher than C. Free superoxide radicals followed the trend of GS3>GS1>C>GS2 and GS6>GS5>C>GS4 and GSM being much higher than C (Fig 6G). Thus, superoxide is the lowest in seedling GS2 and GS4 which has the highest CAT activity and highest in GSM where CAT activity is lowest, indicating that CAT alone can scavenge the superoxide radicals and so there is no need of up-regulation of APX. In *Sorghum*, CAT activity (Fig 6F) was significantly high in SS1, SS2 and SSM and low in SS4 as compared to C. APX activity (Fig 6E) is highest in the control (C>SS3>SS2>SS1and C>SS5>SS4>SS6). No significant change in activity of APX was seen in SSM. Total free superoxide radicals were significantly higher in SS2 and SSM and lower in SS1 and SS4 with respect to that of C (Fig 6G). Thus, SS2 and SSM faced more oxidative stress while SS1 and SS4 faced lesser oxidative stress compared to control. However, there might be some other enzymes such as Glutathione reductase or Superoxide Dismutase involved in scavenging superoxide radicals as there is no clear relationship between CAT/APX and superoxides in *Sorghum* seedlings.

Osmotic stress analyses in terms of total proline and total amino acids (Alanine): In case of *Cicer* seedling, no significant change was observed in total free proline content between control C and the treatments (GS1, GS2, GS3, GS4, GS5, GS6, GSM) (Fig 6H). But there was a difference in total amino acids (Ala), such that amino acid content was in the order of GS3>GS1>C>GS2 and GS5 was significantly higher than control (Fig 6I). In *Sorghum* seedlings, total amino acids were significantly lower than control in the roots of SS2 and SS6 (Fig 6I). Total free proline was significantly higher in roots of SS1 only and in the rest of the samples it was not significant (Fig 6H). Osmotic stress, which is induced by limited water supply, is known to alter the amount of total amino acid contents and proline such that they get accumulated in the plant tissues facing stress [40]. Proline plays a very important role as an osmoprotectant in the adaptation to osmotic stress [41]. The dicot *Cicer* seedlings did not have any significant change in proline accumulation in any sample and only GS1, GS3 and GS5 had higher amount of Ala with respect to control which indicates very little water stress is faced by seedlings growing at 1 ml and 5 ml of <200 nm MSNs and in 2ml of 200-600 nm MSNs. In GS2, the stress was lower than that of Control. In case of monocot *Sorghum*, only SS1 faced osmotic stress, while two seedlings SS2 and SS6 experienced lower stress than control seedlings. This proved that MSNs do not induce significant water stress in either monocot or dicot seedlings and in few doses may lower the stress also.

Effect on Bacillus coagulans: For each dose there is a significant change in growth (Fig 7). Also, there is a significant difference between each treatment at a particular hour. However, there is no significant difference when the interaction between doses and hours were taken into consideration. In Tukey, it was found that there was a significant difference in growth between control and 200 µl and 1000 µl but not between control and 400 µl. Also, when the dose was increased from 200 µl to 400 µl and from 400 µl to 1000 µl there was a significant change in growth. Therefore, it can be deduced that there was no harmful effect of this MSN on *Bacillus coagulans*. Rather it produced a slight increase in growth at 200 µl and 1000 µl concentrations with respect to control.

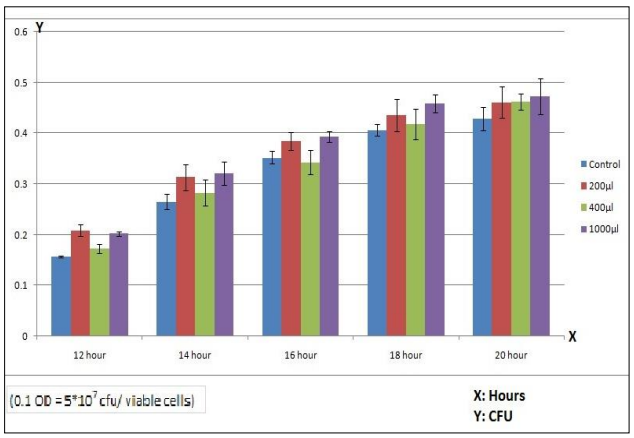

Fig 7 Growth of *B. coagulans* at various concentrations of MSN. N=3. P=0.05.

CONCLUSION

According to the preliminary studies, it can be said that when supplied in controlled dosage, nanoporous silica can modulate morphology, photosynthesis and biochemistry of monocot and dicot plants without inducing huge stress. The <200 nm nanoparticle has been found to increase growth and photosynthesis in model dicot *Cicer arietium* (gram) and decrease the oxidative and osmotic stress without affecting the protein, lipid and carbohydrate content when only 2 ml is applied to 10g soil. However, crude nanoporous silica is found to exert deteriorating effects on photosynthesis and induced oxidative and osmotic stress. In *Sorghum bicolor*, 5 ml of <200 nm particles increased both growth and photosynthesis, 1 ml <200 nm particles lowered oxidative stress and increased photosynthesis but also enhanced osmotic stress, 2 ml and 5ml of <200 nm can be used to increase shootlength. But further studies are required (measurement of SOD and Glutathione reductase) to assert about oxidative stress since APX and CAT did not clearly indicate about oxidative stress. The <200nm and crude nanoporous silica did not exert any harmful effects on the photosynthetic rate of the seedlings. Nanoporous silica also exerted negligible water stress on the seedlings. It also did not harm the gut bacteria and instead increased its growth at certain concentrations over various time scales. More studies are required to ascertain the efficiency of nanoporous silica in modulating biochemical pathways such as respiration, photosynthesis, genomic and proteomic changes to ascertain

its use as novel biofertilizer which are to be carried out as an extension of this study.

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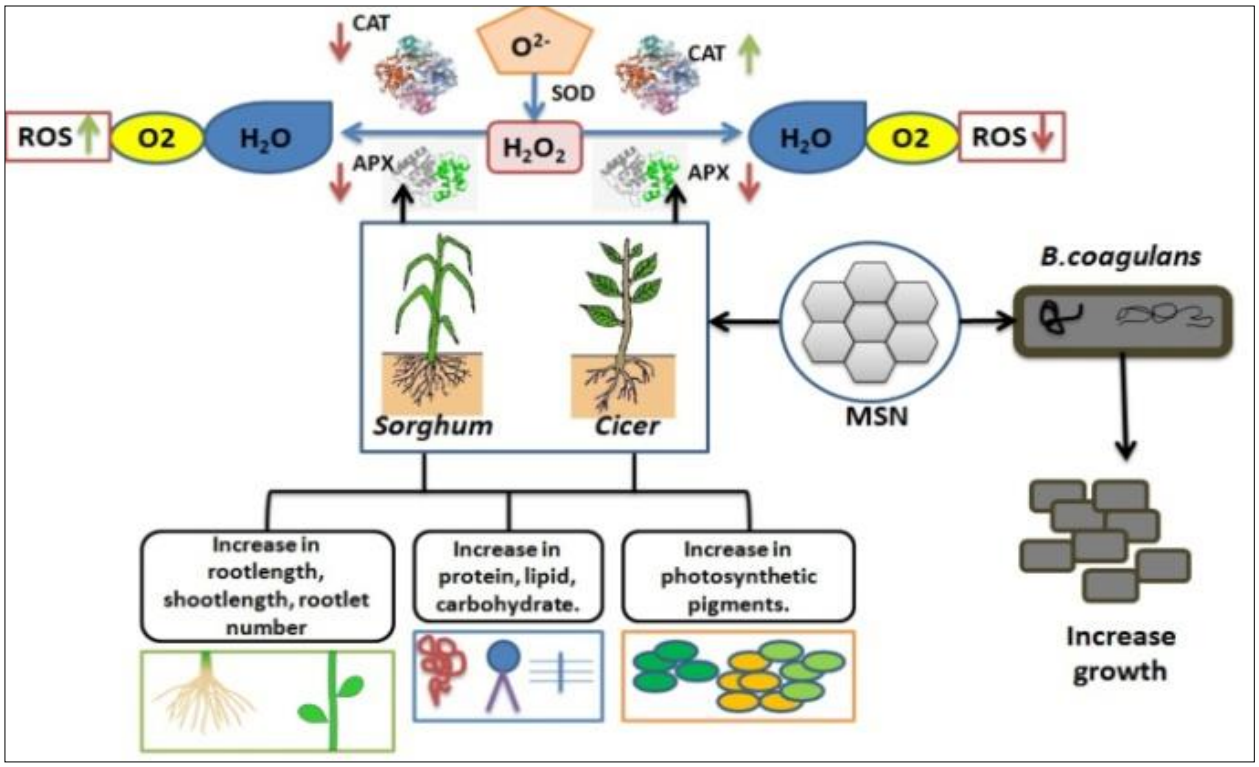
Conflict of interest: *There is no conflict of Interest.*

Ethical aspects: No human or animal samples have been used for the study.

Abbreviations:

MSN, mesoporous silica nanoparticles; APX, Ascorbate Peroxidase; CAT, Catalase; ROS, Reactive oxygen species.



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