SYNTHESIS, CHARACTERIZATION AND APPLICATIONS OF NANOSILICA

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Abstract: The study aimed at evaluating the effect of chemically synthesized nanosilica of different agro-waste sources in biological fields. The nanosilica was synthesized using sol-gel method which provided with approximately 27% yield and progressively characterization of silica nanoparticles was carried out using UV-Visible spectroscopy, FTIR analysis and SEM analysis. In UV-Visible spectroscopy, the four nanosilica samples gave absorption maxima in range of 280-310 nm, FTIR showed presence of absorption peaks of Si-O-Si and Si-O bonds in all four nanosilica samples. The SEM images show spherical nanoparticles ranging from 10-50 nm in size range. The effect of these nanosilica was observed on agricultural and environmental fields such as soil microbial susceptibility, seed germination, resistance against plant pathogenic fungi and prevention algal blooms.

Keywords: Nanosilica, Sol-gel method, Characterization, microbial susceptibility, algal blooms.

I. INTRODUCTION :

An extensive research in the field of nanotechnology and its applications is been carried out in recent years [1,2]. A generalized description of nanotechnology was subsequently established by the national nanotechnology initiative, which defines nanotechnology as the manipulation of matter with atleast one dimension sized from 1 to 100 nanometers. Depending upon the method of preparation, nanorods, nanospheres or nanocapsules can be obtained. Because of their submicroscopic size, they have unique material characteristics, and manufactured nanoparticles may find practical applications in a variety of areas, including medicine, engineering, catalysis, and environmental remediation. Various characterization techniques can image nanoparticles, directly measure sizes, and infer shape information, but they are limited to studying only a few particles at a time. However, those techniques can be quite effective for obtaining basic information about a nanoparticle.

Silica (SiO_2) is one of the valuable inorganic multipurpose chemical compounds. It is the second most abundant molecule on earth's crust [3]. However, manufacture of pure silica is energy intensive. A variety of industrial processes, involving conventional raw materials require high furnace temperatures (more than 700°C). Tetraethoxysilane (TEOS) and tetramethoxysilane (TMOS) has been mainly used as the silica source to produce nanosilica. However, these sources are relatively expensive and exhibit high toxicity [5]. Although a simple chemical process can be used using non-conventional raw materials like rice husk (20-30% silica content), corn cob (60%), rice hay (60-80%) and sugarcane bagasse (88%) for extraction of silica. The sol-gel methods are the most general method of synthesis of silica nanoparticles (SNPs). Nanosilica is an important metal oxide that covers all major fields of science and technology including industrial, electronics and biomedical applications [6]. Appetence in the sol-gel processing of ceramic and glass materials started in the half of 1800s by Ebelman and Graham's researches on silica gels. A solvent extraction process together with the sol–gel technique was employed to prepare spherical Rice husk silica nanoparticles without using templates or surfactants [5,7]. The synthesized nanosilica was characterized by UV Visible Spectrophotometer, Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM).

The introduction of nanoparticles into plants might have significant impact and thus, it can be used for agricultural applications for better growth and yield. Plants generally require silica to control biotic and abiotic stress [8]. The presence of silicon reduces toxic metal elevation and increases water-use efficiency and photosynthesis rate in plants. Generally, several studies are made on toxicity of nanoparticles on seed germination which are based on germination rates obtained with response to nanoparticles. An earlier study shows that the addition of nanosilica in soil enhances growth of maize [5,9]. Even though different sources of silica are used as silicon fertilizers, ecotoxicological properties and the risks of silicon fertilizers in terms of soil microbial health and soil nutrient values are found to be scanty to the best of our knowledge. Thus to evaluate the effect of nanosilica on indeginous soil microbes the different plant growth promoting bacteria (PGPB) and plant growth inhibiting bacteria (PGIB) were tested with the difference sources. Also the effect of the nanoparticles was studied on seed germination of plants like tomato, chilly, wheat and cucumber to see whether it promotes faster germination.

Another mechanism proposed is that soluble silicon acts as a modulator of host resistance to pathogens. Such potential mechanisms have been proposed previously in maize plant. Beneficial role of nanosilica may be feasible to enhance fungal resistance in maize [10]. However, studies regarding the effect of nanosilica on other plant infecting fungus are scarce. The objective of this study is to bring out potential alternatives to bulk silicon sources using nanotechnology, to explore the elusive role of nanosilica in protecting plants against fungal pathogen. Another objective of nanosilica is to decrease the frequency of algal blooms have increased in water bodies. Some of such blooms produce toxins that can cause the death of animals & humans, and more importantly cause the death of aerobic organisms due to the anaerobic conditions, threatens the safety of drinking water and destroy aquatic ecosystem. To reduce and hopefully avoid the potential risks associated with such blooms, several approaches have been developed like addition of algaecide and flocculants. However, various disadvantages have been associated with the practice of using such methods like secondary pollution, low biological selectivity and adverse environmental impacts. Commercially available nanosilica along with a flocculant were used to reduce the cyanobacterial blooms in the lakes [11]. Similarly the nanosilica synthesized from the four sources are tested on algal blooms in a microenvironment to see their effect.

II. MATERIALS AND METHOD:

2.1 Raw materials for silica nanoparticles synthesis.

Silica nanoparticles were synthesized using four agro-waste inexpensive materials which were rice husk, rice hay, corn cob and sugarcane bagasse. Raw materials rice husk and rice hay were collected from rice field where as corn cob and sugarcane bagasse, collected from the local corn seller and juice center at Goregaon station market. (Mumbai, India).

2.2 Synthesis of silica nanoparticles by sol-gel method.

Nanosilica was synthesized by simple sol-gel method [2,5]. A description of sol-gel process can be formation of an oxide network through polycondensation reactions of a molecular precursor in a liquid. The raw materials are treated with acid for 24hrs, filtered and dried, followed by alkali treatment for 24hrs and filtration. This filtrate obtained is titrated with acid to reach a pH suitable for nanoparticle formation. This solution is kept for ageing for 48hrs, centrifuged and kept for drying till amorphous powder is obtained. It is a low cost and eco-friendly method for obtaining mass quantity of nanoparticles.

2.3 Characterization of synthesized nanosilica.

The prepared powder was characterized using UV-Visible spectroscopy (UV-Visible spectroscopy; S.S & L.S Patkar-Varde college, Goregaon, Mumbai, India), Fourier transform infrared spectroscopy (FTIR; Viva college, Virar, Thane, India), and Scanning electron microscopy (SEM; IIT Bombay, Mumbai, India).

2.4 Silica susceptibility test on soil microbes

Selective bacterial cultures namely *Rhizobium* & *Proteus vulgaris* as plant growth promoting bacteria (PGPB) whereas, *Pseudomonas aeruginosa* and *Serratia marcescens* as plant growth inhibiting bacteria (PGIB) were obtained from laboratory of biotechnology, S.S & L.S Patkar-Varde college. Cultures were maintained in Luria-Bertini agar media slants (Himedia, Mumbai, India)

Antibacterial activity was assessed by using strip-diffusion method similar to Kirby-Bauer disk-diffusion method [6] with Mueller-Hinton agar (Himedia, Mumbai, India) as a medium. About 20 ml of sterile molten media was poured into sterile petri plates and allowed to solidify for 5 mins followed by swabbing of 0.1 ml of inoculum (from 24hrs old culture) uniformly over agar. Concentrations of 10,50 & 100 ppm of different nanosilica sources were loaded individually on sterile strips followed by incubation of plates at 37°C for 24 hrs. A control of diluent (sterile saline) and chemically available sodium silicate was used.

2.5 Effect of nanosilica on seed germination

Seeds of cucumber, tomato and chilli were purchased from the local plant nursery in Virar. The seeds were surface sterilized using 5% sodium hypochlorite followed by thorough washing with sterile distilled water. The seeds were placed on sterile petriplates containing filter paper, concentrations of 10, 50 & 100 ppm of the different silica nanoparticles were poured into these plates and the germination rate was observed for 1 week.

2.6 Effect of nanosilica on fungal resistance

Plant pathogenic fungus was isolated from infected leaves of onion. The fungus was enriched in potato dextrose broth and then isolated in potato dextrose agar (PDA) slants. Culture was maintained at 4degree Celcius. The fungal resistance activity of nanosilica was examined by using strip-diffusion method similar to Kirby-Bauer disk-diffusion method, with Mueller-Hinton agar (Himedia, Mumbai, India) supplemented with 2% glucose. About 20 ml of sterile molten medium was poured into sterile petriplates and allowed to solidify for 5 mins, followed by swabbing of 0.1 ml of the fungal spore suspension (from 48 hrs old culture) uniformly over the agar. Concentrations of 10, 50 & 100 ppm of different silica sources were loaded individually on sterile strips followed by incubation of plates at 27°C for 48 hrs. A control of diluent (sterile saline) was used.

2.7 Effect of nanosilica in prevention of algal blooms

The algal sample was obtained from algal bloom affected water body near Borivali station. The chemical PDADMAC was purchased from Just Textile LTD, Ambernath (Thane, India).

The algal sample was enriched in BG-11 broth media. Silica nanoparticle solution of 1000 ppm and PDADMAC solution of 100 ppm was prepared in NaCl solution (0.02). The nanosilica solution was added to the algal culture suspension grown in BG-11 medium, followed by addition of PDADMAC solution to the same suspension [11]. Controls of algal culture suspension with only PDADMAC and algal culture suspension with only nanosilica of the same ppm values were used.

III. RESULTS & DISCUSSION:

3.1 Yield of the synthesized silica nanoparticles.

The synthesis of silica nanoparticles was carried out by sol-gel method using agricultural waste raw materials which is to be considered to be a green synthesis approach.

a) b) c) d)

Figure 1 :- Silica nanoparticles powder of - a) Rice husk, b) Rice hay, c) Corn cob & d) Sugarcane bagasse.

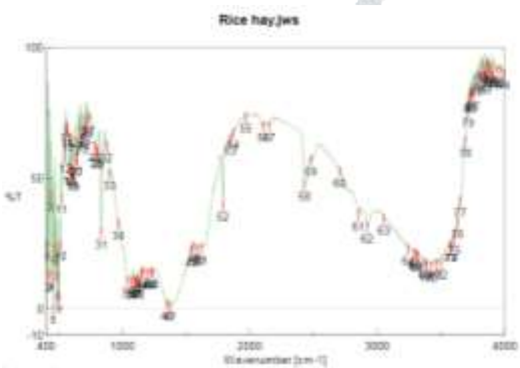
The yield of nanosilica obtained from rice husk was 29.66%, rice hay was 31.62%, corn cob was 21. 89% and that from sugarcane bagasse was 28.12%. Therefore, the total yield obtained was approximately 27%, which shows that the method is suitable for mass production.

3.2 UV-Visible Spectrophotometer analysis.

The absorbance value on UV-Visible spectrophotometer of synthesized nanosilica from rice husk was 305 nm, rice hay was 286 nm, corn cob was 302 nm and sugarcane bagasse was 284 nm. As absorbance all four samples ranged between 280-305 nm which is a closer towards 290-310 nm (the standard range of silica nanoparticles) it indicated that the samples contain silica nanoparticles

3.3 FTIR analysis:

Types of bonds and bond vibrations	Reference standard(cm^{-1})	Rice Husk (cm^{-1})	Rice Hay (cm^{-1})	Corn Cob (cm^{-1})	Sugarcane Bagasse (cm^{-1})
Si-O-Si Asymmetric vibrations	1095	1091.51	1091.51	-	1093.44
Si-O-Si Stretching vibrations	801	798.38	798.38	-	-
Si-O-Si Bending vibrations	474	476.33	-	476.33	473.43
Si-O Stretching vibrations	972	970.01	-	-	-



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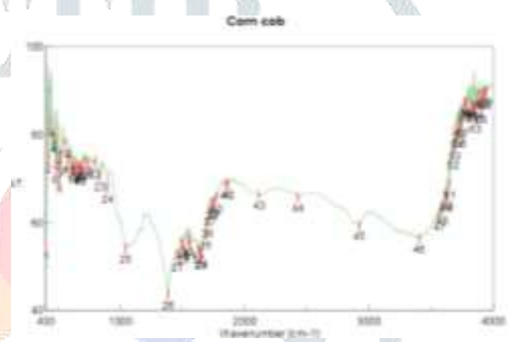

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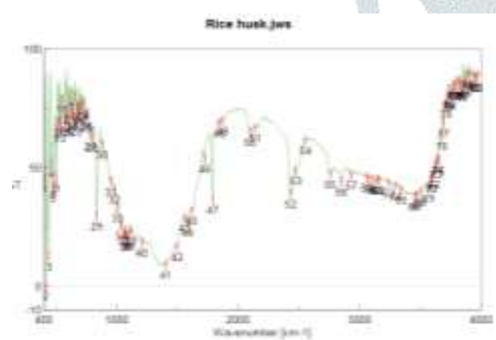

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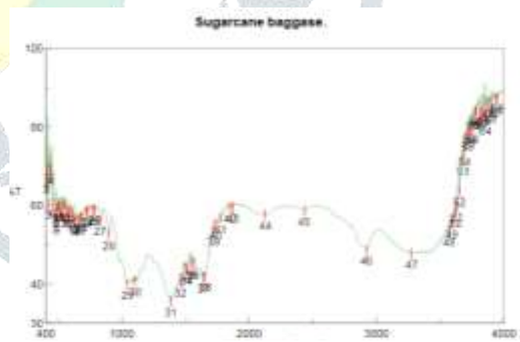

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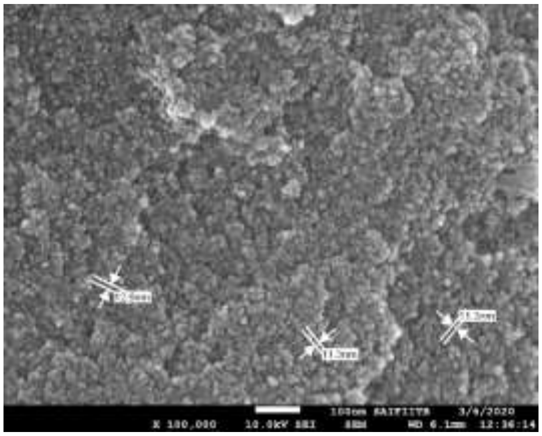
Figure 2:- FTIR analysis graph of - a) Rice hay, b) Rice husk, c) Corn cob & d) Sugarcane bagasse

The FTIR analysis showed presence of siloxane bond (Si-O-Si) asymmetric, stretching & bending vibrations and silanol group (Si-O) stretching vibrations nearer to 1095 & 801 cm^{-1} and 474 & 972 cm^{-1} respectively. Confirming the presence of silica in all four samples.

3.4 SEM analysis:



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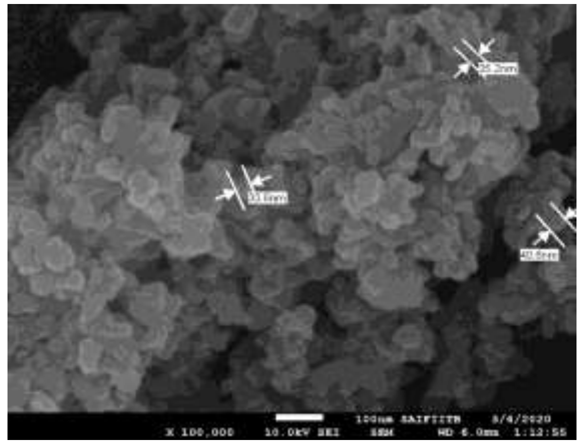

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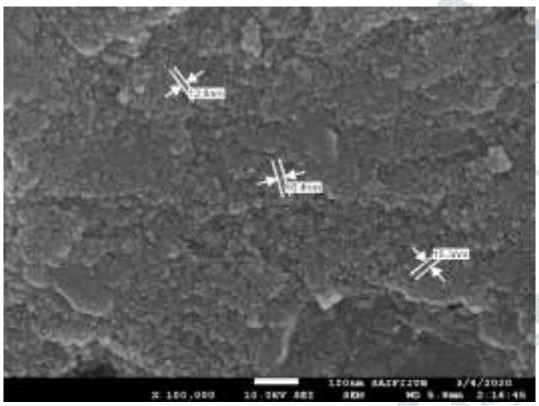

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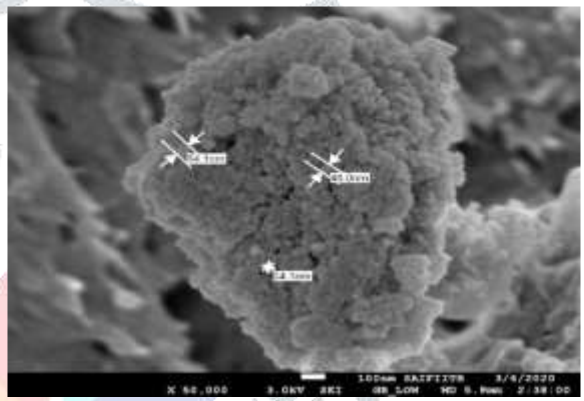

d)

Figure 3:- SEM images of a) Rice hay, b) Rice husk, c) Corn cob, d) Sugarcane bagasse

The topography and morphology of the silica nanoparticles can be seen in the SEM images of all four samples with size range between 10-50 nm in diameter approx. Thus the sol-gel method was efficient with the production of spherical nanoparticles from organic raw materials.

3.5 Microbial susceptibility test:

Figure 4 :- Microbial susceptibility test on soil microbes using nanosilica as a metabolite.

PGPB used were *Rhizobium* and *Proteus vulgaris* and PGIB used were *Serratia spp. and Pseudomonas aeruginosa*. The activity of nanosilica was checked at concentrations 10, 50 and 100 ppm against the bacteria along with the control (saline). There was neither a promoting nor an inhibiting effect was seen on the PGIB and PGPB by all the four samples.

3.6 Resistance against plant pathogenic fungi4 :

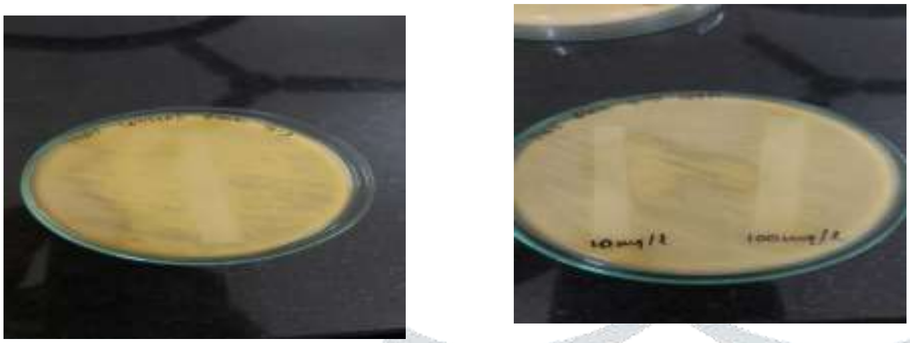

Figure 5 :- Antimicrobial susceptibility test on pathogenic fungi using nanosilica as an antimetabolite

The antifungal activity of silica nanoparticles was carried out against *Aspergillus niger* by strip diffusion method. The activity of silica nanoparticles from all four sources was checked at 10, 50 and 100 ppm concentrations along with the control (saline). The control showed excessive growth as well as spore formation throughout the plate after 48 hours of incubation. Hence, the NPs do not have inhibitory effect on the growth of *Aspergillus niger*.

3.7 Seed Germination:

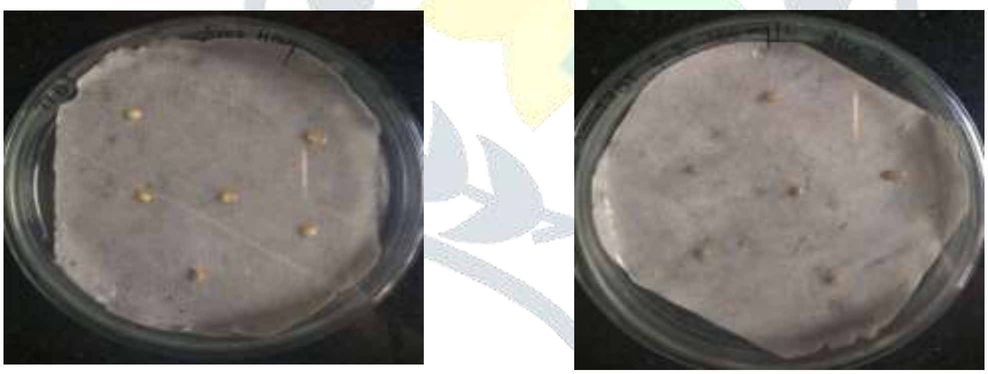

Figure 6 :- Estimation of seed germination rate using nanosilica as a nutrient.

The seeds used for determination of the germination rate efficiency were chilli, tomato and cucumber seeds. When compared with the control, rice hay and sugarcane bagasse showed higher germination rate at the lowest silica nanoparticles concentration. Rice hay also showed faster germination of tomato at high silica NPs concentration. Chilli showed growth at high concentration of corn cob silica NPs. However when the plants root and shoot length were compared with respect to control, chilli showed good growth at all silica NPs lowest concentration. Tomato showed good growth at the silica NPs highest concentration.

3.8 Algal bloom prevention:

Figure 7 :- Nanosilica along with PDADMAC used for algal bloom trearment.

There were many tubes set such as only culture suspension in BG11 medium, culture suspension mixed with PDADMAC, four individual controls containing culture suspension and silica nanoparticles solutions. The aggregation and settlement of algal cells was seen in the test samples. PDADMAC helps to accumulate the cells and silica NPs increase the density of aggregate leading to deposition of the algal cells at the bottom of tube within 30 minutes.

4 CONCLUSION:

This study demonstrated that sol-gel method was efficient in synthesizing amorphous silica nanoparticles from all the four agro waste sources in range of 10-50 nm size range when analysed by UV-Visible spectroscopy, FTIR analysis and SEM analysis. The average yield obtained from all sources was 27% of total raw material. From applications performed, the silica nanoparticles at concentration between 10 to 100 ppm concentrations can be applied for agricultural applications maintaining the biodiversity of soil microflora as well as these concentrations of nanosilica might enhance the growth rate of certain seeds. However, upto 100 ppm concentration of the synthesized nanosilica has no inhibitory effect on the growth of plant pathogenic fungal species. These nanoparticles along with a flocculant help in reduction of algal blooms thus might be beneficial if used for controlling eutrophication in water bodies. It became evident with the help of applications performed that nanosilica synthesized is non-toxic towards environmental microflora thus it can be applied in environmental applications. The purified form of this nanosilica might be more efficient in its activity in a particular field

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