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Synthesis and Characterization of Silica Nanoparticles from Sorghum Straw (*Sorghum bicolor (L.) Moench*) and the Evaluation of its Effect on Seed Germination of *Beta vulgaris* (Beetroot)

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Abstract

The synthesis of silica nanoparticles (Si-NPS) using the sol-gel method and characterization, as well as their impact on the germination of Beta vulgaris seeds were investigated. Si-NPs were synthesized using sorghum straw, characterized using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy, SEM/Energy Dispersive Spectroscopy, X-ray Spectroscopy (EDS). Assessment of the results carried out on the synthesized Si-NPs revealed the following: FTIR showed absorption bands at 790.20, 1051.10, 1636.30, and 3403.10 cm^{-1} indicating the presence of Si-H, Si-O-Si, Si-O and Si-OH respectively. XRD analysis showed that the Si-NPs had an average size of 30.46 nm. SEM analysis showed that the Si-NPs were made up of amorphous mesoporous structures. The elemental composition of the sample was found to be 65.33 % Si, 12.77 % O, 11.05 % Al, 5.23 % Fe, 3.42 % C and 2.20 % Na. The result from the germination study demonstrated that as the concentration of Si-NPs increased from (0-8 g/L), the germination rate and vigour index of seeds also increased, with 8 g/L having the highest germinated seedlings. The control which has no Si-NPs recorded the least seedling growth. The study of the germination time also showed that seeds with the highest Si-NPs concentration of 8 g/L, germinated within the least germination period (2 days) when compared with the germination period of the control. This research has shown that increase in concentration of Si-NPs have a positive effect on the germination of Beta vulgaris (Beetroot).

Keywords: *Beta vulgaris* (Beetroot), Characterization, Silica Nanoparticles, Sorghum Straw, Synthesis

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INTRODUCTION

Nanotechnology is used to solve numerous environmental challenges resulting from air pollution, water pollution and solid waste disposal. Its use has become necessary due to global human activities and lifestyle changes. These changes result in the release of various types of toxic non-degradable inorganic/organic materials from activities such as open-air burning of unwanted agricultural waste, dumping of industrial effluents into water bodies, carbon monoxide emissions by vehicles etc (Taran *et al.*, 2020). According to the World Bank, waste production rates have increased in recent years and are projected to rise significantly to about 2.2 billion tons per year in 2025 (The World Bank Group, 2021). Thus, the production of Si-NPs from agricultural waste such as sorghum straw could play a vital role in solving biotic and abiotic stress in plants (Goswami *et al.,* 2022). The main factor influencing crop productivity and development is biotic stress. Abiotic stress is brought about by things like climate change, nutrient deficiency, heavy metal exposure and water stress. Reactive oxygen species buildup in a plant results in the harmful effects of abiotic stress on plants (Rajput *et al.,* 2021; Xu *et al.,* 2015).

Nanoparticles are small materials with sizes ranging from 1 to 100 nm. Due to their small sizes, they have enhanced properties such as very large surface area, stability and unique optical characteristics (Anubhav & Gupta, 2021). Synthesis of nanoparticles from agricultural waste, through eco-friendly and simple methods may have the potential to improve germination, act as plant growth regulators, pesticide or fertilizer delivery systems, and wastewater treatment methods. Nanoparticles large surface area makes them attractive to solve agricultural and environmental challenges. Inorganic nanoparticles such as SiO_2, ZnO, TiO_2, and Al_2O_3 have also been reported to have favourable effects on plant growth (Ghidan & Al Antary 2020). A nanoparticle is an excellent nutrient ion carrier due to its exceptional qualities, which include a large surface area and superior water absorption rate. In general, plants have strong defence in place to deal with stress (Rhaman *et al*., 2022).

Silica nanoparticles can also give plants resistance, by acting as a regulator to safeguard plants from abiotic stress. This is done by creating a double layer of epithelial cell walls within the plant, boosting the antioxidant defence system and lessening the damaging effects of stress. This can be accomplished by nano-priming seeds to deposit Si-NPs on the surface of plants (Goswami *et al.,* 2022).

"Sorghum straw", which is a byproduct as well as a waste after sorghum cultivation, is rich in silica. Its potential as a silica precursor remains largely untapped, leading to environmental pollution (Athinarayanan *et al.,* 2020) as such, this research is channeled towards the utilization of the waste sorghum straw into something meaningful and to reduce environmental pollution.

In this context, we investigate the conversion of sorghum straw into Si-NPs, which hold various applications in biotechnology and agriculture. Additionally, the effects of these Si-NPs on seed germination on an important plant crop: "beetroot" (*Beta vulgaris*) was evaluated. This study will contribute to the development of novel and sustainable nanotechnology based strategies for improving the production and quality of beetroot, as well as other leafy vegetables.

MATERIALS AND METHODS

Area of study

MATERIALS

The materials used includes sorghum straw (stalks, leaves and panicles) gotten from Igabi, Kaduna state Nigeria. *Beta vulgaris* seeds were purchased from Faluchi Agro Company, Lagos State, Nigeria. The reagents that were used in this research are sodium hydroxide (99% British Drug House reagent grade, CAS Reg.No.56-81-5), Hydrochloric acid (Analar reagent grade 35% Assay, Density-1.18 kg/L, CAS Reg.No.7647-01-0), and Sodium hypochlorite (Epochem-SH, 13 % w/v), distilled water was used to prepare all solutions.

METHODS

Collection of samples

Sample Pretreatment/Preparation

The sorghum straw were washed first with tap water to remove sand and other particles, then rinsed with distilled water and sun-dried for 5 hrs in a strainer. The sample was further pulverized with a vibrating cup mill and sieved using a 60 mm mesh sieve to obtain fine powder. Exactly 50 g of the sample was subjected to pretreatment by soaking the sorghum sample in 500 cm^3 of 2 M HCl for 1 hr and afterwards, the excess liquid was decanted. The sorghum sample was dried in an oven for 12 hrs at 90 °C to remove moisture. The sorghum straw sample was calcinated by controlled burning at 650 °C for 4 hrs in a muffle furnace. This was done to burn all organic compounds as they would interfere with the subsequent analysis. The resulting Sorghum Straw Ash (SSA) was left overnight to cool in the furnace and then used for further analysis. (Buás de Lima *et al.,* 2011; Bello *et al.,* 2018; Sapawe & Hanafi, 2018).

Silica Nanoparticles Extraction

The Si-NPs were extracted from the SSA sample, by pouring 100 cm^3 of 2 M NaOH solution to the ash in (plate 1) a 250 cm^3 beaker. For a total of 2 hrs, the SSA solution was stirred continuously on a magnetic stirrer plate at a steady 90 ^oC to dissolve the silica into a sodium silicate solution (Azat *et al.,* 2019). The solution was filtered with a whatman (No.1) filter paper and the insoluble residue discarded. The sodium silicate (filtrate) was allowed to cool at room temperature. Exactly 130 cm^3 of 2 M HCl was carefully added drop wise to the sodium silicate solution to lower the pH from 14-6.5 using a pH meter. At pH 6.5, the silica was precipitated into xerogels. The precipitated silica from the sodium silicate solution was allowed to age for 24 hrs to allow the growth of silica particles (Buás de Lima, *et al.,* 2011; Bello *et al.,* 2018). The precipitate was then filtered and washed severally with distilled water to remove sodium chloride residue. Lastly, the precipitated gel was dried in an oven at 105 ^oC to constant weight (Azat *et al.,* 2019). The hardened silica was grinded to powder using a mortar and pestle (plate 1). The Si-NPs was weighed and stored in an airtight container for further analysis.

CHARACTERIZATION OF THE SYNTHESIZED SI-NPs

The Si-NPs were characterized using the Fourier Transform Infrared spectrometer (FTIR-Agilent technologies Cary-630), X-Ray Diffractometer (Zeiss Auriga M12-051), Scanning Electron Microscope (Empyrean Joel Japan), Energy Dispersive X-ray Spectroscopy (Empyrean Joel Japan).

Germination Test Procedure

Exactly 30 seeds of *B. vulgaris* (10 seeds for each replication) were selected and the surface sterilized with 500 cm^3 of 10 % sodium hypochlorite solution by soaking them for 10 minutes. This procedure was done to eliminate microorganisms present on the surface of the seeds. The excess 10 % NaOCl solution was decanted and the seeds were rinsed three (3) times with distilled water before transferring into petri dishes (Siddiqui & Al-Whaibi, 2014).
The different Si-NPs solutions prepared were used for seed treatment. Exactly 30 seeds of each plant (10 seeds for each replication) were soaked in 100 cm^3 distilled water (control) and 100 cm^3 of each Si NP solution, shaken gently and kept for 2 hrs. After two hours, the soaked seeds were removed from the mixture solution via decantation (Parveen & Rao 2015).
Table 1: the number of seeds distributed for various concentrations of Si-NPs dispersion

Concentration	No of seeds distributed
Si-NPs	***B.vulgaris***
Control	30
2 g/L	30
4 g/L	30
6 g/L	30
8 g/L	30
Total	150

Seed Germination Test

Exactly 10 seeds of each concentration of Si-NPs were selected and evenly spaced on towel paper moistened with distilled water .The towel paper with seeds was then placed in 15 mm × 100 mm petri dishes. Finally, the dishes were sealed with a plastic wrap and lids respectively, and kept on a lab bench. This was done at normal laboratory conditions for seed germination to commence (Chourasiya *et al.,* 2021). The experiment lasted for 16 days, and was performed in triplicate. At every four days intervals, the germinating seedlings were transferred onto filter papers and germination parameters were assessed. Fifteen (15) seedlings of each plant were randomly selected for data recording (Acharya *et al.*, 2020).

Germination Percentage

Germination percentages were determined by recording the number of seeds that developed a primary root of at least 2 cm in length (Siddiqui & Al-Whaibi, 2014). The seeds were then set aside for further observation. The germination rate was calculated by dividing the number of germinated seeds by the total number of seeds tested. (Parveen & Rao 2015). Final germination percentage was calculated using the formula adopted by Alsaeedi *et al.,* (2011).Eqn 1:

$$FGP = \left[\frac{TNG}{TNP}\right] \times 100 \quad (1)$$

Where,
FGP: final germination percentage,
TNG: total number of germinated seeds &,
TNP: total number of planted seeds.

Mean germination time

The mean germination time was calculated by formula used by Ranal & De Santana (2006) Eqn 2:

$$MGT = \frac{\sum_{i=1}^{k} NiTi}{\sum_{i=1}^{k} Ni} \quad (2)$$

Where,
Ti = time from the start of the experiment to the ith interval,
Ni = number of seeds germinated in the ith time interval and,
K = total number of time intervals.

Vigor index (VI)

Vigor index (VI) was calculated using equation 3:

$$VI = \left\lceil \frac{SDM(g) \times GP}{100} \right\rceil \quad (3)$$

Where, VI = vigor index,
SDM = seedling dry mass (g) and,
GP = germination percentage (Alsaeedi *et al.*, 2011).

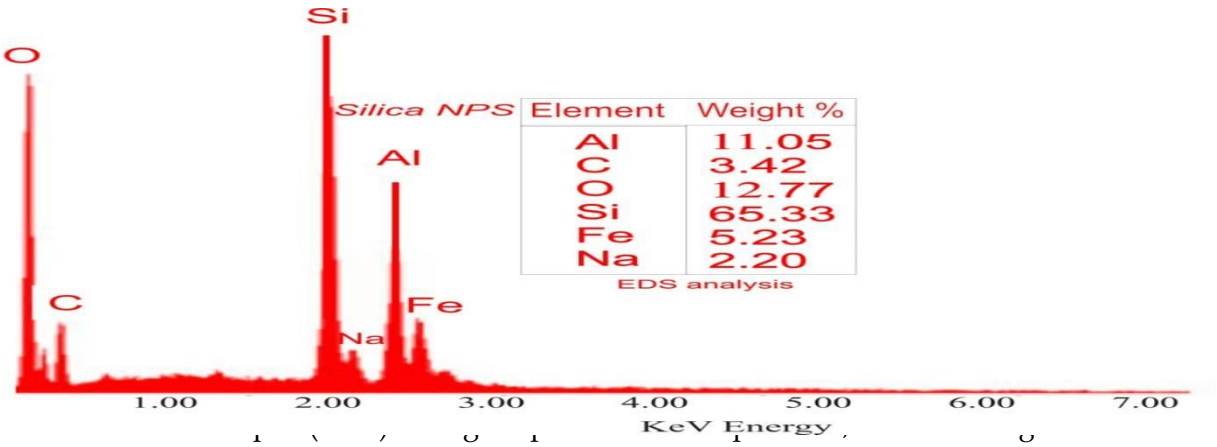
Seedling dry weight

Exactly 5 seedlings of each replication of the plant seeds were harvested, placed in a crucible, and then dried in an oven maintained at 80°C for 24 hours. The seedlings were kept in a desiccator overnight to cool. This process was repeated until a constant weight was achieved after three measurements. The measurements were made using an analytical weighing balance. It was done in order to obtain the dry weight of the seedling (Chourasiya *et al.*, 2021).

RESULTS AND DISCUSSION

Energy Dispersive Spectroscopic Investigation

Energy dispersive spectroscopic (EDS) images were obtained at an acceleration voltage of 0 - 7 keV. Fig 1. shows results in terms of weight percentage. EDS analysis gave 65.33% Si and 12.77% Oxygen, along with trace amounts of sodium (2.20%), aluminum (11.05%), carbon (3.42%), and iron (5.23%), which indicates the presence of impurities (Waseem *et al.*, 2009).



magnifications of 8000 x. SEM micrographs showed that Si-NPs were spherical in shape. The SEM micrographs showed that the particles have a heterogeneous mesopore structure, with

varying grain sizes and distinct dark and bright areas. The dark areas are pore cavities, while the bright areas are consistent with those of amorphous Si-NPs. They also show evidence of agglomeration, which is consistent with the results obtained in previous reports by Supiyani *et al*., (2022); Patil *et al*., (2018).

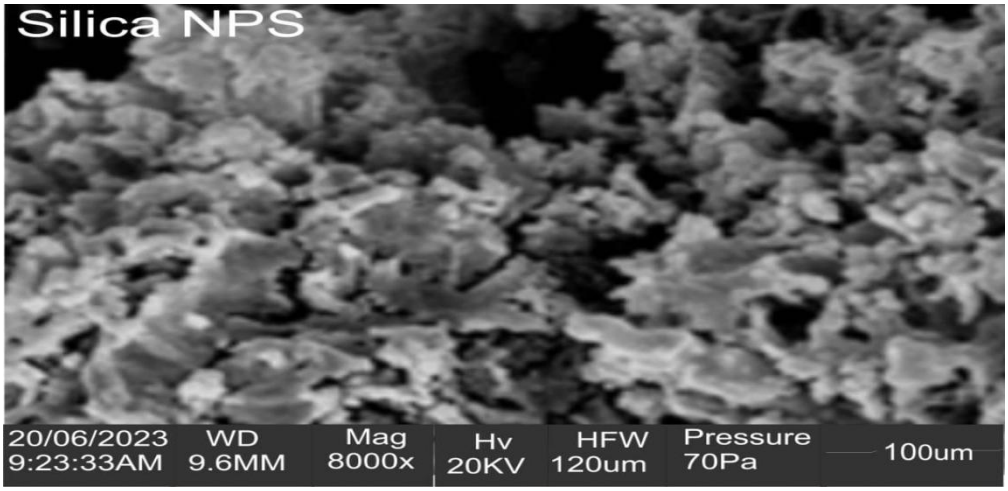


Plate 1. Scanning Electron Microscope **Image of the Si-NPs at 8000×Mag**

FTIR Analysis of Si-NPs

Fourier Transformed Inra-red (FTIR) spectroscopy was used to identify the functional groups present on the surface of the Si-NPs in the spectral range of 650-4000 cm^{-1}. The peaks in the spectrum (Figure 2.0) indicate the presence of certain functional groups. The typical absorption bands of the Si-NPs were at 790.20, 1051.10, 1636.30, and 3403.10 cm^{-1}. The Si-OH symmetric stretching and OH from the absorption of water molecules were responsible for the broad band at 3403.10 cm^{-1}. The spectrum also revealed a strong peak at 1051.10 cm^{-1}, which is consistent with Si-O-Si bending vibrations. The peak at 790.20 cm^{-1} is consistent with the Si-H stretching, which indicates that the sample contains hydrogen-bonded silanol groups. The peak at 1636.30 cm^{-1} can be attributed to Si-O stretching vibration of the gel network. This confirms that the sample is composed of Si-NPs. It also agrees with previous studies reported by, Patil *et al*., (2018), Mehmood *et al*., (2019); Supiyani *et al*., (2022).

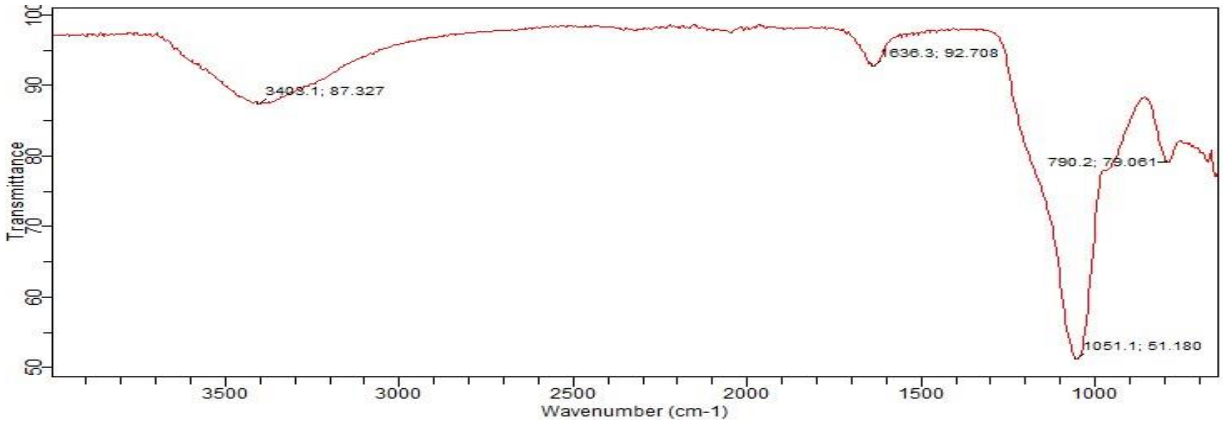


Fig 2. Fourier Transformed Inra-red **Spectrum of Si-NPs**

X-Ray Diffractometer (**XRD) Analysis of the Si-NPs**

The X-Ray Diffractometer analysis of the synthesized Si-NPs showed diffraction peaks at 2θ = 29.00°, 32.26°, 41.11°, 46.00°56.98° respectively (Fig. 3.) The average particle size was calculated using the Debye-Scherrer in equation 4.1.The average particle size of Si-NPs was 30.46 nm. The XRD pattern of the synthesized Si-NPs shows strong diffraction peaks at 2*θ*. The high intensity of the peak at 56.98 shows the Si-NPs had a well-defined structure. The similarity of the diffraction peaks to those reported in previous studies by Suriyaprabha *et al.* (2012); Goswami & Mathur, (2022) confirmed the amorphous structure of the Si-NPs.

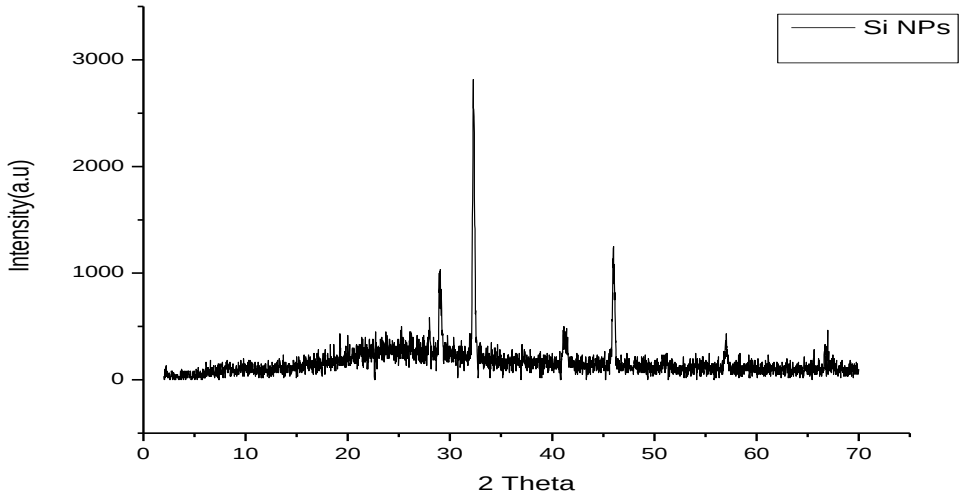


Fig 3. X-Ray Diffractometer **Spectrum of the Synthesized Si-NPs**

The Effect of Si-NPs on Seed Germination of *Beta vulgaris*

Table 2.0 present the germination parameters calculated for *Beta vulgaris*. All treatments were found to exhibit higher final germination percentages and vigour indexes than untreated seeds (plate 3.). For beetroot seeds, there was a significant difference in germination time between treatments with different Si-NPs concentrations. Seeds treated with 6 and 8 g/L Si-NPs concentrations had a shorter germination time of 2 days (Fig.4.). However, those treated with lower concentrations of 2 g/L and the control solution had a longer germination time of 16 days. The results indicate that the higher the Si-NPs concentration, the greater the germination percentage. According to Lu *et al.,* (2015), tomato germination increased by about 70 % after applying Si-NPs suspension with 5 mg/L. Similarly, an increase was found in maize seed germination after treatment with Si-NPs (Suriyaprabha *et al.*, 2012; Mushtaq *et al.*, 2017). Plant productivity depends on genetic and environmental factors, but seed germination plays a vital role in determining a plant's yield. The seeds treated with Si-NPs had a higher vigour index (VI) than those in the control solution. A treatment of 8 g/L resulted in the highest measured VI (Fig.5.). A higher vigour index means the seedlings are stronger when there is a higher concentration of Si-NPs. A study conducted by Alsaeedi *et al.*, (2019), also found that cucumber seeds treated with Si-NPs had a high vigour index. VI is a key characteristic of seed germination, as it determines and specifies whether the seedlings will continue to grow well after germination or not. The results of this study are consistent with those published by Azimi *et al.*, (2014), which showed that adding Si-NPs by 40 mg/L increased tall wheatgrass vigor index by more than 100 %. Lu *et al.*, (2015) also found that treating tomato seeds with 7 g/L of Si-NPs had similar effects. *B. vulgaris* seeds showed different mean germination time (MGT). MGT of

seeds was decreased when Si-NPs were applied. In contrast to control seeds, which took 12-16 days to germinate, seeds treated with 8 g/L Si-NPs only needed 4-8 days (Fig.6.). Additionally, Si-NPs treatments with 2, 6 and 4 g/L significantly reduced mean germination time compared to control seeds. Furthermore, Suriyaprabha *et al.*, (2012) found that Si-NPs positively decreased MGT of maize seeds. The results were also confirmed by Azimi *et al.*, (2014), who reported that adding Si-NPs by 40 mg/L reduced tall wheat grass seed's germination time significantly.

Plate 3. Germination Seedlings of *Beta vulgaris* with Si-NPs control, 2, 4, 6 and 8 g

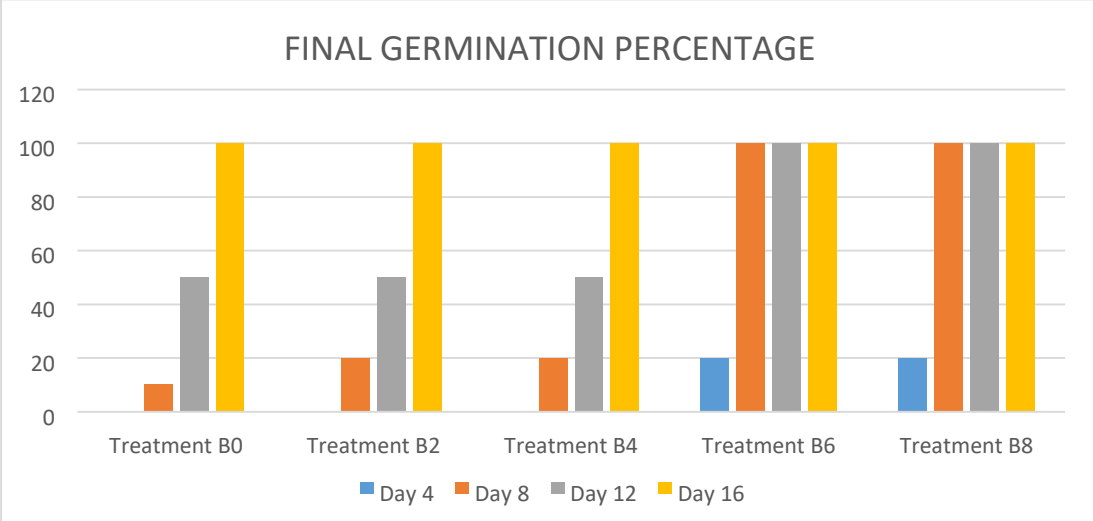


Fig. 4.0 Effect of Si-NPs on the Final Germination of *Beta vulgaris*

Table 2.0 Results of the effect of germination on *Beta vulgaris* seed.

COS	TNP	TNG				MGT				FGP				VI			
		4	8	12	16	4	8	12	16	4	8	12	16	4	8	12	16
0	10	-	1	5	10	16	8	2	1	-	10	50	100	-	0.0007	0.0036	0.0071
2	10	-	2	5	10	16	4	2	1	-	20	50	100	-	0.0014	0.0035	0.0070
4	10	-	2	5	10	16	4	2	1	-	20	50	100	-	0.0016	0.0040	0.0080
6	10	2	10	10	10	2	1	1	1	20	100	100	100	0.0019	0.0095	0.0095	0.0095
8	10	2	10	10	10	2	1	1	1	20	100	100	100	0.0092	0.0096	0.0096	0.0096

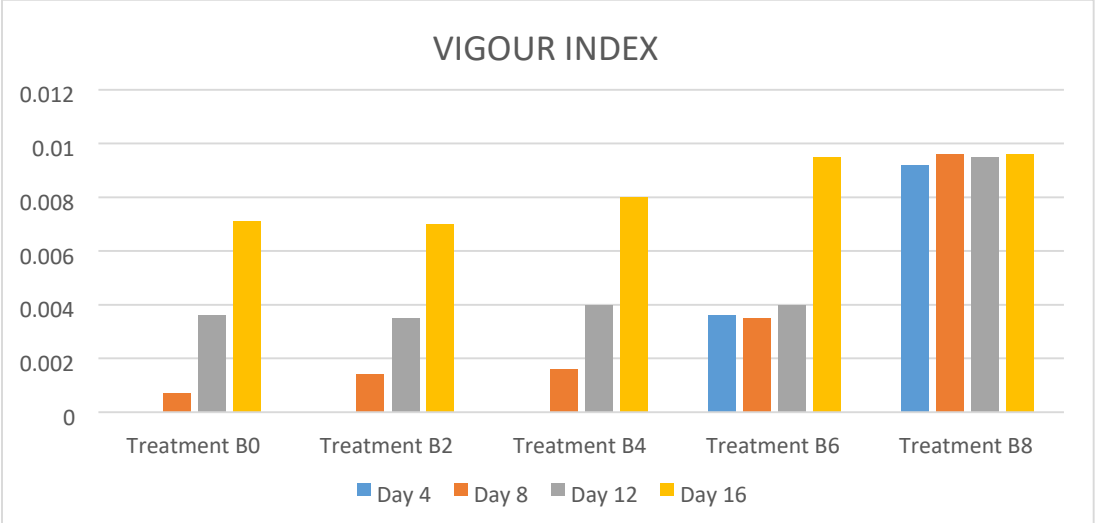


Fig. 5.0 Effect of Si-NPs on the Vigour Index of *Beta vulgaris*

Key: MGT = Mean Germination Time/Day, FGP = Final germination Percentage, VI; Vigor Index, TNG = Total number of Seeds Germinated/Day, TNP = Total Number of Planted seeds, COS = Concentration of Si-NPs (g/L)

CONCLUSION

This research synthesized Si-NPs using the sol-gel method. A positive assessment was obtained from FTIR, XRD, and SEM/EDS analyses carried out on the synthesized Si-NPs. Results of the characterization also showed that just like rice husk, corn cobs and sugarcane bagasse, silica from sorghum straw have beneficial properties for agricultural uses. This research evaluated the potential effect of Si-NPs derived from sorghum straw as a simple and effective fertilizer. The data obtained from the germination tests showed that Si-NPs produced from sorghum straw had a positive effect on the germination rate, vigour index and mean germination time of seeds when compared to the control. This suggested that Si-NPs synthesized from sorghum straw could be used as an effective fertilizer for agricultural production, as it has the potential to increase crop yields.

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