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Seed priming of fenugreek seeds with silica nanoparticles: enhanced growth economics and sustainable agricultural practices

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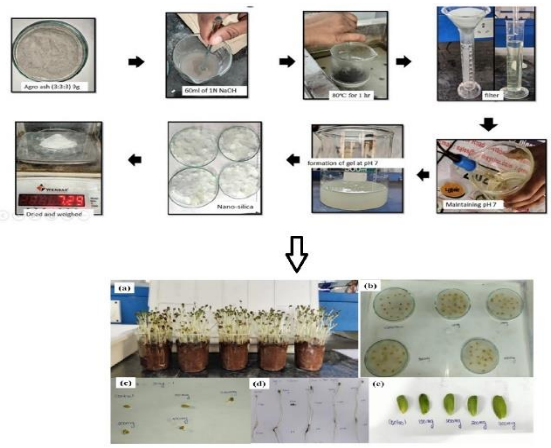
Graphical abstract



Abstract

Background: This study aimed to evaluate the impact of various concentrations of nano-silica on the germination process of fenugreek seeds. The agro-waste materials such as rice husk, corn cobs and bagasse were used as a precursor for synthesizing nano-silica. Moreover, their formation was confirmed by assessing their structural phase formation, elemental composition and functional group. For seed priming of fenugreek seeds, nano-silica concentrations of 100 – 400 mg/L were utilized.

Results: The agro-waste-derived nano-silica was crystalline, with 46.37 nm in size and a surface area of 361.706 m^2/g. The germination percentages of all fenugreek seedlings were intensely enhanced by applying nano-silica priming. The nano-silica-treated fenugreek seedlings showed 68.5% germination during day one, reaching their maximum during day 4. It also showed increased root length (8.6 -10 cm), shoot length (6.2 - 7 cm) and leaf size (1.71 - 3.08 cm^2) compared with control. Furthermore, it positively influenced fenugreek seeds' silica, chlorophyll, and polyphenol content.

Conclusion: The findings indicated that treating the fenugreek seeds with nano-silica could enhance their germination by boosting the antioxidant system, thereby safeguarding the plants against oxidative stress. Nevertheless, additional investigations are necessary to ascertain the hazardous impact of nano-silica concentration before its widespread production and use in agricultural applications.

Keywords: Fenugreek seeds; germination rate; growth economics; nano-silica; seed priming

1. Introduction

Plants are undeniably a substantial provider of nourishment for both humans and animals through the production of essential molecules (i.e., carbohydrates, proteins, and lipids). The germination process is a vital stage in a plant's life cycle, marking the growth initiation from a seed to a young plant. However, slow or unsuccessful germination can significantly impact plant development and viability (Sembada and Lenggoro, 2023; Sembada and Faizal, 2022). Multiple investigations and research on enhancing seed sprout rates and seedlings' quality were investigated to address this issue. Despite the diligent efforts of scientists, it was unable to achieve successful output enhancement. This investigation aimed to devise strategies and approaches to expedite germination or overcome obstacles to dormancy, resulting in improved and effective seedling production. By accelerating germination, plants can gain early access to nutrients and gain a competitive advantage in growth. The quality of seedlings is crucial for their long-term success and resilience to unfavorable environmental conditions. Various techniques, such as water soaking, immersion in saline/osmotic solutions, plant growth regulators, and chemical compounds, can stimulate germination and improve seedling quality (Sembada and Lenggoro, 2023; Javed *et al*. 2020; El-Sanatawy *et al*. 2021). However, this study explores seed priming techniques using nanoparticles to accelerate germination and enhance seedling production.

Nanotechnology has become increasingly prevalent in various health, aerospace, and industrial sectors. Moreover, it has the potential to revolutionize global food production in agriculture significantly (Saritha *et al*. 2022). Previous research suggests that nanoparticles can be utilized as a fertilizer by releasing modest amounts of metal ions to enhance plant nutrition or improve plant tolerance, promoting their growth. Moreover, nano fertilizers avoid nutrient leaching and volatilization, preserving soil fertility longer than traditional fertilizers (Jiang *et al*. 2022). Several researchers have focused on creating natural, biomaterial-based, and environmentally acceptable priming agents to guarantee ecological integrity (Hameed *et al*. 2020). Seed priming is a notable strategy for improving seed germination and positively influencing the early growth phases of plants. This approach provides advantages such as enhanced ability to withstand stress, increased strength and vitality of young plants, successful germination of less robust seeds, improved quality of seeds, and higher agricultural productivity. Moreover, it is a sustainable agricultural practice that enhances farmers' socio-economic welfare (Acharya *et al*. 2020).

Various articles have provided evidence of the beneficial impact of seed priming with nanoparticles on seed germination. These nanoparticles demonstrated a beneficial impact on the seed germination process, particularly when the seeds were subjected to high levels of salt stress. Nano-priming therapy can enhance germination percentage, rate, seed microstructure, and antioxidant enzyme activity (Maroušek *et al*. 2022). Nano-priming can significantly reduce input costs for farmers by improving seed germination and plant growth, reducing the need for fertilizers and pesticides. This leads to cost savings over the growing season. Additionally, it can lead to higher crop yields, resulting in higher revenues for farmers. Furthermore, enhanced seed performance and reduced chemical treatments can decrease labor costs for crop management, further reducing overall costs (do Espirito Santo Pereira *et al*. 2021. This study explores silica nanoparticles' use to expedite the germination process of fenugreek seeds.

Silicon has long been overlooked for its role in plant biology. While it is not considered an essential element for plants, it can accumulate in plants at similar levels to other essential macro elements. Recent research has shown that silicon can help mitigate the adverse effects of various stresses on plants, including salt and drought stresses, metal toxicity, diseases, pests, radiation damage, high temperatures, and nutrient imbalances (Jiang *et al*. 2022; Ivani *et al*. 2018; Siddiqui *et al*. 2014). Seed Priming employing silica nanoparticles improves seed germination and plant growth, reducing chemical fertilizer and pesticide use. It may reduce soil and water pollution (Hasanaklou *et al*. 2023). Comparatively, other sustainable agricultural practices like organic farming, regenerative agriculture, and agroforestry provide well-established benefits but come with challenges and trade-offs. For instance, 'Organic farming' promotes biodiversity and soil health and often has lower yields and higher labor costs. Similarly, 'Agroforestry' integrates trees and shrubs into agricultural landscapes, enhancing biodiversity, improving soil health, and sequestering carbon; however, it requires significant initial investment and skilled expertise and may take several years to see economic returns (https://www.ceew.in/publications/sustainable-agriculture-india?form=MG0AV3)

Several studies have reported on the successful output of nano-silica for germination enhancement. Siddiqui and Al-Whaibi (2014) applied 12 nm nano-silica to enhance tomato seeds' germination, while Ivani *et al*. (2018) applied 10-15 nm nano-silica for the extended germination period of Feenugreek seeds. Studies have also shown that nano-silica treatment is more effective in maize expression than bulk silica, making it a potential alternative for sustainable farming (Suriyaprabha *et al*. 2014). Additionally, nano-silica has been found to protect wheat seedlings from oxidative and photosynthesis damage and is more effective in reducing UV-B stress than bulk silica due to its greater availability (Tripathi *et al*. 2017). However, the nano-silica utilized for seed priming was reportedly synthesized through chemical routes. Nevertheless, this research focused on synthesizing the silica nanoparticles utilizing agro-based waste materials like rice husk, corn cobs and bagasse, which might remain a sustainable approach.

Fenugreek is a highly valued herb used for medicinal purposes since ancient times. It possesses various beneficial properties such as sedative, carminative, diuretic, and antimicrobial effects. Fenugreek contains essential components like diosgenin and saponin, contributing to its anti-diabetic and antioxidant properties (Patel *et al*. 2021). The cultivation of fenugreek can not only enhance farming systems but also improve human health. It is crucial to implement scientific strategies such as breeding programs and cultural applications to achieve high yields of fenugreek. Different agricultural practices can impact the quality characteristics of fenugreek, from sowing to harvesting. Factors like sowing times, fertilizer applications, and stress conditions can positively and negatively affect the plant's quality properties (Tabassum *et al*. 2021). The present research focused on improving the growth economics of fenugreek seeds through seed priming techniques using nano-silica synthesized sustainably. Their germination rate, root and shoot length, and leaf size were assessed to establish the effectiveness of seed priming on fenugreek seeds. Moreover, their chlorophyll content, silica content and polyphenol content were also evaluated.

2. Experimental section

2.1. Materials used

Analytical chemicals of sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), sodium hypochlorite (NaClO), Folin-Ciocalteu reagent and hydrochloric acid (HCl) are used in this study. Rice husk, Corn cobs and bagasse were collected from local shops in Coimbatore, Tamilnadu, India. The fenugreek seeds were purchased from the local markets of Coimbatore, Tamilnadu, India. Then, the seeds were sterilized with NaClO solution for 10 min (Goswami *et al*. 2022).

2.2. Synthesis of agro-waste-derived nano-silica

The agro-wastes (i.e., rice husk, corn cob, bagasse) were washed with distilled water to remove any adhering dirt and sun-dried for one day. Each agro-waste were ground mechanically into a fine powder and calcinated in a muffle furnace at 600 °C for two hours; thus, agro-waste ashes were prepared. Equal weights of agro-waste ashes (i.e., 3:3:3) were mixed homogenously and agitated with 60 mL NaOH (1 N) at 80 °C. The resultant solution was filtered, and the filtered solution was cooled down to room temperature. An appropriate quantity of HCl (1 N) was added till it reached to pH of 7 and constantly stirred. Then, it was incubated for 12 hr to commence gel formation. The synthesized gel was desiccated for 24 hr at 80 °C to obtain nano-silica and then dried at 80 °C (Goswami *et al*. 2022). Thus, agro-waste-derived nano-silica (i.e., AWNS) was synthesized (**Figure 1**).

2.3. Characterization analysis

The agro-waste-derived nano-silica was assessed with FTIR (JASCO 4100, Japan) to identify their functional groups over the 400-4000 cm^{-1} wavenumber region. The XRD (Bruker D8, Germany) with CuK_{α} radiation source was used to analyse their phase formation, and the Sorption analyzer (Quantachrome, USA) determined their surface area characteristics. Their morphological characteristics and elemental composition were visualized from SEM (ZEISS) and EDAX (Bruker).

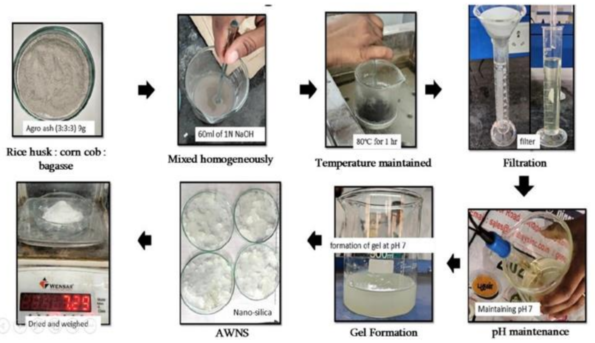


Figure 1. Synthesis of Agro-waste-derived nano-silica

2.4. Seed priming of fenugreek seeds with AWNS

The sterilized fenugreek seeds were treated with AWNS suspension of various concentrations (100 – 400 mg/L), denoted as T100, T200, T300 and T400; the distilled water served as a blank (CS). The seeds were soaked in these concentrations for 4 hr before plantation (Alsaeedi *et al*. 2019). The effect of AWNS on the fenugreek seeds was assessed through their germination rate, leaf size and physiological and biochemical analysis. The AWNS-treated seeds in various concentrations were placed in a Petri plate with cotton as a base and grown under laboratory conditions at room temperature (i.e., 24 ± 2 °C) in a dark condition. The germination rate was estimated based on the number of seeds germinated, and seeds with a root tip of 1 mm or greater were considered germinated (Alsaeedi *et al*. 2019). After germination, the AWNS-treated seeds in various concentrations were planted in soil in a beaker and placed under laboratory conditions at room temperature (i.e., 24 ± 2°C). Their respective root and shoot lengths were recorded during the 3rd, 6th and 9th day (Alsaeedi *et al*. 2019).

2.4.1. Biochemical analysis

On the ninth day, the leaves from each test sample were assessed for their total silica content, chlorophyll content and total polyphenol content.

The protocol for total silica content determination: the leaves from each test sample were collected, rinsed with deionized water, and dried at 105 °C for 20 min. Then, it was crushed in a mortar pestle and homogenized with 5 mL sodium hydroxide (40 %) and 5 mL water. Afterward, it was placed in a sterile pot and subjected to 121°C for 20 min. Subsequently, 5 mL sulphuric acid (5 M) and then 40 mL water was added. Finally, the processed samples were measured for silica content by molybdenum blue calorimetry (Du *et al*. 2022).

The protocol for chlorophyll content determination: 0.1 g of leaves from each test sample were weighed and ground in a mortar pestle with 10 mL acetone (80 %). Then, it was centrifuged at 5000-10000 rpm for 5 min. The protocol was repeated until the residue's colour disappeared. Then, the absorbance of the solution was measured at 610 and 550 nm with acetone as blank (Kumari *et al*. 2018). Finally, the concentration of chlorophyll a (Ch_a), chlorophyll b (Ch_b), and total chlorophyll (Ch_{Total}) were calculated using the Eq. (1)-(3)

$$Ch_a\left(mg/g\right)=\left(12.7*A_{610}\right)-\left(2.59*A_{550}\right) \quad (1)$$

$$Ch_b\left(mg/g\right)=\left(22.9*A_{610}\right)-\left(4.7*A_{550}\right) \quad (2)$$

$$Ch_{Total}\left(mg/g\right)=\left(8.2*A_{610}\right)-\left(20.2*A_{550}\right) \quad (3)$$

The protocol for total polyphenol content determination: 0.5 g of leaves from each test sample was extracted into 20 mL ethanol (95%), homogenized well, filtered, and the filtrate was used for the subsequent analysis. Then, 1 mL Folin-Ciocalteu reagent and 0.8 mL Na2CO3 (7.5%) were added. Finally, the absorbance of the sample was recorded at 765 nm against blank samples. The results were expressed in mg equivalent of Gallic acid (GAE) per g dry weight (Geremu *et al*. 2016; Rahmani *et al*. 2018)

2.4.2. Statistical approach

Data obtained from the experimental studies were statistically approached using Microsoft Excel 2021determining the mean values and standard deviation. Experiments were randomly designed with three replications for each treatment. Moreover, the results were analyzed using a One-way ANOVA test.

3. Results and discussion

3.1. Characterization analysis

3.1.1. Crystallographic and surface area features of AWNS

Figure 2(a) displayed the XRD diffractogram of AWNS nanoparticles over the 2θ range from 20°–80°. Although the nano-silica was prepared using agro-waste materials like rice husk, corn cob and bagasse, the resultant nano-silica formed possessed a crystalline phase, depicting sharp diffraction peaks. Therefore, it confirmed the successful employment of agro-wastes. Based on the peaks observed at 2θ = 31°, 47°, 57° and 68° indicates the presence of cubic silica nanoparticles. These 2θ values correspond to the JCPDS card no: 00-026-1481 representing the following 'hkl planes' presented in **Figure 2(a)**. The absence of a peak at 2θ = 22° further confirms the crystallographic characteristics of silica nanoparticles (Daulay *et al*. 2022) Hence, the nano-silica formation was successful. The Debye-Scherrer equation (i.e., d = 0.9λ/βcosθ) (Jayalakshmi *et al*. 2021) was employed to determine the average crystallite size of AWNS, which was 46.37 nm.

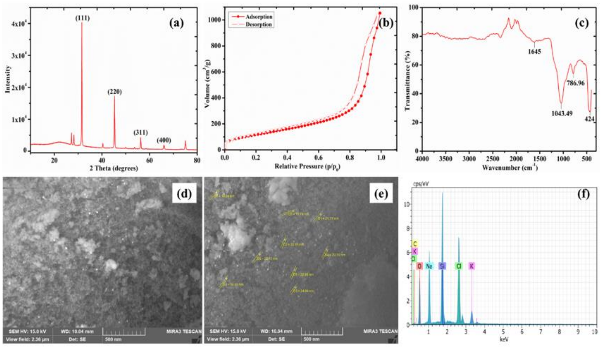


Figure 2(a). XRD of AWNS; (b) Adsorption/desorption curve of AWNS; (c) IR spectrum of AWNS; (d) & (e) SEM micrograph of AWNS at various spots; (f) Elemental mapping of AWNS

The BET analysis determined the surface area characteristics of AWNS extracted from the combination of the agro-waste mixture. **Figure 2(b)** portrayed the hysteresis loop displaying Type IV isotherm with an H_3 hysteresis loop. Moreover, the hysteresis loop showed that AWNS was wedge-shaped and primarily comprised of mesopores. Furthermore, the surface area of AWNS was 361.706 m^2/g, which is higher than Silica nanoparticles synthesized using various sources. Idris *et al*. (2023) synthesized nano-silica using corn cobs and olive stones that yielded 270 and 300 m^2/g, respectively (Idrs R *et al*. 2023). Similarly, Rovani *et al*. (2019) reported a surface area of 63 m^2/g for nano-silica synthesized from sugarcane waste (Rovani *et al*. 2019). The pore distribution of AWNS revealed that the average pore radius was 15.489 Å, and the total pore volume was 1.578 cm^3/g. Moreover, their pores were distributed between 1.5 to 5.0 nm.

3.1.2. Functional group of AWNS

Figure 2(c) represents the FTIR spectrum of AWNS, depicting the most prominent peaks and types of bonding vibrations. It revealed the consecutive absorption peaks at 1645, 1043.49, 786.96 and 424 cm^{-1} (Maroušek *et al*. 2022). The oxide group of AWNS (i.e., Si-O-Si bending vibrations) was detected at a frequency of 424 cm^{-1}. An asymmetric stretch of the Si-O-Si bond was detected at a peak of 1043.49 cm^{-1} (Ruiz *et al*. 2024). The peak at 786.96 cm^{-1} denoted the symmetric stretching vibrations of Si-O-Si (Ruiz *et al*. 2024). The weaker bonds are visualized between 2400 – 2200 cm^{-1} due to the stretching vibrations of AWNS. The peak at 1645 cm^{-1} represents the characteristic of the Si-OH bond (Imoisili *et al*. 2020; Yadav *et al*. 2019).

3.1.3. Morphological features and elemental composition of AWNS

The morphology of AWNS extracted from the combined agro-waste is depicted in **Figure. 2(d) and (e)**. The SEM images (**Figure. 2(d) and (e)**) represented the morphology of nano-silica observed from various spots, while **Figure. 2(e)** represented the particle size distribution. **Figure. 2(d)** revealed the non-uniform-sized distribution of silica nanoparticles. Their particle distribution was 18.24 – 24.84 nm. The AWNS exhibited regularly shaped particles with smooth surfaces. The AWNS had spherical morphology with no aggregation (i.e., the AWNS particles have remained separated). Therefore, the nano-silica prepared using the agro-wastes was successful. The purity of AWNS synthesized from a combination of agro-wastes was confirmed by EDAX analysis. **Figure. 2(f)** exhibited the EDAX spectrum of AWNS, which displayed the signal peaks for Si (18.33%), O (35.66%), Na (15.83%), Cl (21.11%), K (4.74%) and C (4.34%). The peaks related to Si and O suggested the successful formation of silica nanoparticles. The EDAX spectrum also revealed the presence of impurity elements (i.e., Na, Cl and K), which resulted from processing the nanoparticles with NaOH and HCl during the AWNS synthesis process.

3.1.4. Toxicity analysis on AWNS

The AWNS's toxicity was assessed using the DXY-2 Microtox test device, determining the bioluminescence intensity. A freeze-dried *Vibrio fischeri* (i.e., luminescent bacteria) was mixed with sodium chloride in an ice bath for 2 minutes and served as standard. It was then exposed to mercury chloride (i.e., blank) and AWNS solution (i.e., 100 – 500 mg/L) for 30 minutes. The bioluminescence intensity was measured, and a linear equation was framed connecting the bioluminescence intensity to the mercury chloride concentration, which was then correlated with its toxicity. The mercury chloride exhibited a bioluminescence intensity of 50.26%, whereas AWNS showed 85.65%, indicating low toxicity towards *V. fischeri*. Therefore, the AWNS of concentration 100 to 500 mg/L was non-toxic and could be applied for seed priming. Moreover, prior research indicated that nano-silica exhibited toxicity only at elevated doses. Furthermore, nano-silica's biological activity in bacteria is due to their membrane characteristics. The nano-silica may readily infiltrate bacterial cells and engage with lipid membranes, instigating the production of reactive oxygen species that induce biomolecule peroxidation, establishing a toxicological mode against *V. fis*cheri (Ríos *et al*. 2018; Wang *et al*. 2016).

3.2. Effect of AWNS on fenugreek seed germination

Table 1 represents the analysis of growth patterns of AWNS-treated fenugreek seeds (T100, T200, T300, T400) and control seedlings (CS). The values represent the mean ± standard deviation at a significance of 5%. The growth enhancement could be noticed with a lower concentration (T100) than the control; however, the higher concentration of AWNS (T400) significantly enhanced the seed germination, shoot length, root length and leaf size. The seed germination was higher (68.75%) for T300 and T400 during day 1, and they reached their highest level of seed germination during day 4. The potential mechanism that contributed to the higher proportion of seed germination: due to their hydrophilic nature, nano-silica might have facilitated the imbibition process in fenugreek seeds, accelerating their ability to absorb and retain water (Sembada and Lenggoro, 2023; Singh *et al*. 2021). The increased water absorption capability in fenugreek seeds could have fastened their germination rate, reducing the germination period.

Table 1. Growth Analysis of Fenugreek Seeds

Treatment	AWNS concentration (mg/L)	Seed germination (%)	Root length (cm)	Shoot length (cm)	Leaf size (cm^2)
T100	100	62.50	8.6 ± 0.2	6.2 ± 0.2	1.71 ± 0.1
T200	200	62.50	9.5 ± 0.1	6.4 ± 0.1	1.90 ± 0.2
T300	300	68.75	9.7 ± 0.2	6.7 ± 0.1	2.20 ± 0.1
T400	400	68.75	10 ± 0.1	7 ± 0.2	3.08 ± 0.2
CS	Distilled water	56	6.3 ± 0.2	5 ± 0.1	1.44

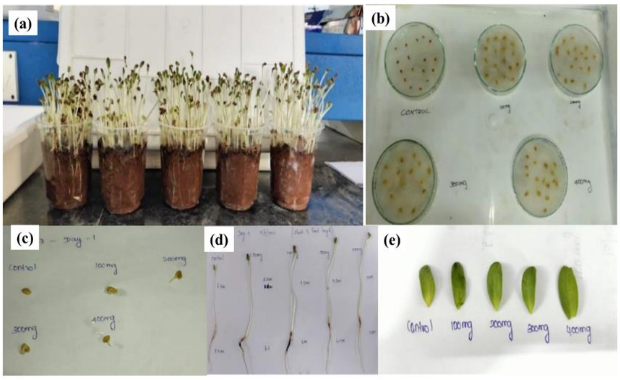


Figure 3. Seed priming with varied AWNS concentrations (a) Fenugreek seedlings; (b) Number of seeds germinated; (c) Germination rate at day 1; (d) Shoot and root length at day 9 and (e) Leaf development at day 9

Fenugreek seedlings' root and shoot lengths were notably more extraordinary in T400 (400 mg/L AWNS), while the T100 ((100 mg/L AWNS)) had the lowest germination rate; however, it is comparably higher than control seedlings. The shoot and root lengths increased significantly with AWNS concentrations on fenugreek seeds. Root elongation with seed priming may be due to the nano-silica's small size and extensive surface area, which enabled them to penetrate the roots directly, thereby enhancing root growth (Jiang *et al*. 2022; Feist *et al*. 2018). Moreover, the growth of fenugreek leaves exhibited a similar pattern. The 400 mg/L AWNS enhanced the biochemical metabolism, improving seed germination and root, shoot and leaf development. The biochemical metabolism might not have enhanced with a lower concentration of AWNS, resulting in minimal seed germination. Furthermore, the application of AWNS increased the elongation of the root, shoot, and leaf size.

Figure 3(a) depicted AWNS-treated fenugreek germinant's fresh and dry weight. When used in the growth medium, adding AWNS nanoparticles to fenugreek seeds considerably improved the germination percentage (GP), enhancing all the vegetative parameters. The error bar represents the mean ± standard deviation values at a significance of 5%. Almost all the AWNS-treated fenugreek seeds had higher fresh and dry weight values than untreated seeds (CS). The presented data shows that the T200 achieved the highest fresh and dry weight values, recording 0.317 and 0.022 g per germinant, respectively. However, the difference between other AWNS treatments (i.e., T100, T300, and T400) was insignificant.

Figure 3(b) depicted AWNS-treated fenugreek seedlings' root dry weight (RW_{dry}). The error bar represents the mean ± standard deviation values at a significance of 5%. After 21 days, AWNS-treated seedlings experienced enhanced RW_{dry} compared to control seedlings. Significantly, RW_{dry} values increased when fenugreek seeds were treated with 100 mg/L AWNS (i.e., T100). Moreover, the T100 recorded the highest RW_{dry} (0.143 g per plant) among all other treatments, including the control. Beyond this concentration (> 100 mg/L AWNS), a gradual decrease was observed in the RW_{dry} values; however, it was still higher than CS. A similar trend was observed for shoot dry weight (SW_{dry}), illustrated in **Figure 3(b)**. However, the maximum SW_{dry} value of 0.120 g per plant was observed with T200. Also, the SW_{dry} values tend to decrease for T300 and T400. The least SW_{dry} of 0.077 g per plant was observed with CS. Similar findings have been reported, stating that nano-silica treatment increased tomato and cucumber seedlings' fresh and dry weights (Alsaeedi *et al*. 2019; Siddiqui *et al*. 2014).

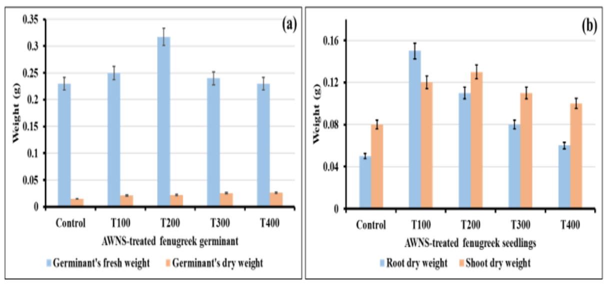


Figure 4. AWNS-treated fenugreek seeds (a) Fresh and dry weight of germinant; (b) Dry weight of the root and shoot of seedlings

3.3. Effect of AWNS on chlorophyll content in Fenugreek seedlings

The enhancement of AWNS-treated fenugreek seedlings was assessed for their photosynthetic features and are presented in **Figure 4**. Following the treatment with the AWNS nanoparticles, the levels of Ch_a, Ch_b and Ch_{Total} in the leaves were analyzed to determine their photosynthesis and plant growth further. Leaves obtained from AWNS-treated pots revealed that the Ch_a, Ch_b and Ch_{Total} contents increased substantially with elevated AWNS concentrations and time (**Figure. 5(a)**). The error bar represents the mean ± standard deviation values at a significance of 5%. The Ch_a, Ch_b and Ch_{Total} contents were maximum for T400 and lowest for CS. Chlorophyll a was 4.469 mg/m^2 (T400) and 3.310 mg/m^2 (CS); Chlorophyll b was 8.043 mg/m^2 (T400) and 5.959 mg/m^2 (CS); total chlorophyll was 14.478 mg/m^2 (T400) and 9.574 mg/m^2 (CS). During photosynthesis, the plants typically generate 'reactive oxygen species' (Xiong *et al*. 2021). Consequently, plants require antioxidant mechanisms to counteract this stress response. The increased photosynthetic traits generated with a higher concentration of AWNS are most likely aided in overcoming the oxidative stresses, resulting in growth enhancement of fenugreek (Jiang *et al*. 2022).

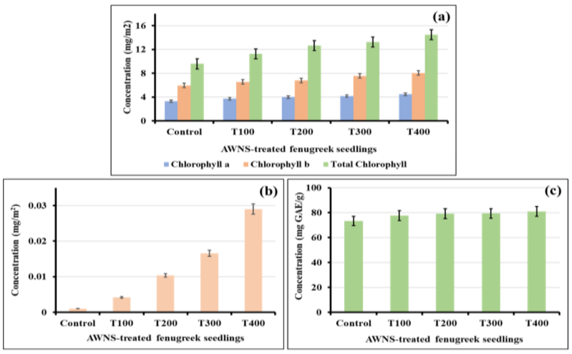


Figure 5. AWNS-treated fenugreek seedlings (a) Chlorophyll content, (b) Silica content, and (c) Polyphenol content

3.4. Effect of AWNS on silica content in fenugreek seedlings

Estimating silica content helps determine the silica uptake ability of fenugreek seeds. Therefore, leaves obtained from AWNS-treated and control pots were assessed for silica content, and the respective results are presented in **Figure 5(b)**. The error bar represents the mean ± standard deviation values at a significance of 5%. The silica content significantly increased with higher concentrations of AWNS and longer time intervals. The silica absorption was highest for T400 (0.0029 mg/m^2) and lowest for CS (0.0014 mg/m^2). The presence of silica content in CS may be attributed to releasing smaller quantities of soluble silica into the solution. This observation reveals that due to their nano-sized particles, the AWNS might have favoured silica absorption and enhanced early seedling growth in fenugreek seeds.

3.5. Effect of AWNS on total polyphenol content in fenugreek seedlings

The total polyphenol content (TPC) determines the 'antioxidant system', an innate protective mechanism that safeguards cells in plants when external factors trigger them. It aids in overcoming 'reactive oxygen species' and 'lipid oxidation' in cell membranes. Moreover, it guarantees a protective barrier to protein and nucleic acid damage during oxidative stress. Therefore, leaves from AWNS-treated and control pots were analyzed for TPC. The TPC values increased significantly with escalating AWNS concentrations and expanding time intervals (**Figure 5(c)**). The error bar represents the mean ± standard deviation values at a significance of 5%. Polyphenol content was highest for T400 (80.976 mg GAE/g) and lowest for CS (73.264 mg GAE/g). These findings highlighted the positive impact of AWNS nanoparticles' treatment with fenugreek seedlings on their antioxidant system and their influence on the total polyphenol content.

3.6. Cost analysis and practical challenges

The cost breakdown of nano-silica prepared using agro-waste materials is depicted in **Table 2**. The expected cost required for preparing AWNS was 690 per kg.

Table 2. Cost breakdown for AWNS preparation

S.No	Particulars	Cost/kg
1	Raw Materials	Nil
2	Washing cost	3.39
3	Blending cost	2.38
4	Stirring cost	6.79
5	Drying cost	164
6	Chemical cost	450
Net cost per kg	626.56	
10 % of the overall cost	62.65	
Total cost Estimated per kg	689.21	

The farmers might bear the initial expenditure for implementing nano-priming technology, such as preliminary expenditure in apparatus, training, and materials in relation to the prospective long-term savings and enhanced profitability. Moreover, the efficacy of nano-priming is contingent upon market acceptability, customer preferences, and the demand for crops cultivated using this method. Furthermore, it is important to ensure the adherence of the nano-priming process to local agricultural norms and standards to prevent legal and financial complications.

4. Conclusion

Nanoparticles have been extensively utilized in agriculture due to their well-documented advantages. The yield of crops is primarily dependent on germination, as successful germination assures an appropriate number of plants per unit of cultivated land. Furthermore, the robust seedling ensures optimal growth despite biotic and abiotic stresses. The present study demonstrated the significance of nano-silica derived from agro-waste in promoting the germination of fenugreek seedlings. AWNS's chemical composition (EDAX) and functional groups (FTIR) have confirmed nano-silica formation. The XRD pattern of AWNS exhibited prominent diffraction peaks at 2θ angles of 36.01°, 32.11°, 46.11°, and 57.13°. The crystalline structure was validated, and the average particle size was determined to be 46.36nm.

All the fenugreek seeds treated with nano-silica exhibited superior and elevated values of G_P. Out of the seed priming treatments (i.e., T100, T200, T300, and T400), the T400 had the most positive impact on all aspects of germination and growth. The seed priming of fenugreek seeds with 400 mg/L AWNS resulted in the highest final G_P, germination speed and lowest mean germination time. Similarly, the vegetative parameters, including RW_{dry} and SW_{dry}, as well as the relative water content, were shown to be higher when treated with 400 mg/L AWNS. Also, the silica and polyphenol content was maximum for 400 mg/L nano-silica, which enhanced early seedling growth in fenugreek seeds. Based on the aforementioned results, it can be inferred that utilizing 400 mg/L AWNS is crucial for the germination and growth of fenugreek to optimize its productivity. The positive and beneficial impact of nano-silica can be ascribed to its small dimensions, enabling it to readily traverse the cell membrane and stimulate numerous physiochemical processes that enhance germination and growth.

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Reference

Acharya P., Jayaprakasha G.K., Crosby K.M., Jifon J.L. and Patil B.S. (2020). Nanoparticle-mediated seed priming improves germination, growth, yield, and quality of watermelons (Citrullus lanatus) at multi-locations in Texas. *Scientific reports*. **10**(1), 5037.

Alsaeedi A.H., Elgarawany M.M., El-Ramady H., Alshaal T. and Al-Otaibi A.O.A. (2019). Application of silica nanoparticles induces seed germination and growth of cucumber (Cucumis sativus). *Meteorology, Environment and Arid Land Agriculture Sciences*, **28**, 57–68.

Daulay A. and Gea S. (2022). Synthesis Si nanoparticles from rice husk as material active electrode on secondary cell battery with X-Ray diffraction analysis. *South African Journal of Chemical Engineering*, **42**, 32–41.

do Espirito Santo Pereira A., Caixeta Oliveira H., Fernandes Fraceto L. and Santaella C. (2021). Nanotechnology potential in seed priming for sustainable agriculture. *Nanomaterials*, **11**(2), 267.

Du J., Liu B., Zhao T., Xu X., Lin H., Ji Y., Li Y., Li.Z., Lu C., Li.P., Zhao H., Li.Y., Yin.Z. and Ding X. (2022). Silica nanoparticles protect rice against biotic and abiotic stresses. *Journal of Nanobiotechnology*, **20**(1), 197.

El-Sanatawy A.M., Ash-Shormillesy S.M., Qabil N., Awad M.F. and Mansour E. (2021). Seed halo-priming improves seedling vigor, grain yield, and water use efficiency of maize under varying irrigation regimes. *Water*. **13**(15), 2115.

Feist B. and Sitko R. (2018). Method for the determination of Pb, Cd, Zn, Mn and Fe in rice samples using carbon nanotubes and cationic complexes of batophenanthroline. *Food chemistry*, **249**, 38–44.

Geremu M., Tola Y.B. and Sualeh A. (2016). Extraction and determination of total polyphenols and antioxidant capacity of red coffee (Coffea arabica L.) pulp of wet processing plants. *Chemical and Biological Technologies in Agriculture*, **3**, 1–6.

Goswami P. and Mathur J. (2022). Application of agro-waste-mediated silica nanoparticles to sustainable agriculture. *Bioresources and Bioprocessing*. **9**(1), 9.

Hameed A., Khalid A., Ahmed T. and Farooq T. (2020). Nano-priming with Zn-chitosan nanoparticles regulates biochemical attributes and boost antioxidant defence in wheat seeds. Agrochimica: *International Journal of Plant Chemistry, Soil Science and Plant Nutrition of the University of Pisa*: **64**(3), 2020, 207–221.

Hasanaklou N.T., Mohagheghi V., Hasanaklou H.T., Ma'mani L., Malekmohammadi M., Moradi F. and Dalvand Y. (2023). Seed nano-priming using silica nanoparticles: effects in seed germination and physiological properties of Stevia Rebaudiana Bertoni. *Chemical and Biological Technologies in Agriculture*, **10**(1), 96.

https://www.ceew.in/publications/sustainable-agriculture-india?form=MG0AV3

Idris I., Naddaf M., Harmalani H., Alshater R. and Alsafadi R. (2023). Efficacy of olive stones and corncobs crystalline silica nanoparticles (Sio2, NPs) treatments on potato tuber moths (Phthorimaea operculella). *Silicon*. **15**(8), 3591–3598.

Imoisili P.E., Ukoba K.O. and Jen T.C. (2020). Green technology extraction and characterisation of silica nanoparticles from palm kernel shell ash via sol–gel. *Journal of Materials Research and Technology*, **9**(1), 307–313.

Ivani R., Sanaei Nejad S.H., Ghahraman B., Astaraei A.R. and Feizi H. (2018). Role of bulk and Nanosized SiO_2 to overcome salt stress during Fenugreek germination (Trigonella foenum-graceum L.). *Plant signaling & behavior*. **13**(7), e1044190.

Javed T., Ali M.M., Shabbir R., Gull S., Ali A., Khalid E., Abbas A.N. and Tariq M. (2020). Rice seedling establishment as influenced by cultivars and seed priming with potassium nitrate. *Journal of Applied Research in Plant Sciences*. **1**(2), 65–75.

Jayalakshmi R. and Jeyanthi J. (2021). Spectroscopic investigation of carbon nanotube as nano-filler entrapped in chitosan hydrogel beads. *Journal of Molecular Structure*. **1237**, 130386.

Jiang Y., Yang J., Li M., Li Y., Zhou P., Wang Q., Zhang P., Rui Y., Lynch I. (2022). Effect of silica-based nanomaterials on seed germination and seedling growth of rice (Oryza sativa L.). *Nanomaterials*. **12**(23), 4160.

Kumari R., Ashraf S., Bagri G.K., Khatik S.K., Bagri D.K. and Bagdi D.L. (2018). Extraction and estimation of chlorophyll content of seed treated lentil crop using DMSO and acetone. *Journal of Pharmacognosy and Phytochemistry*, **7**(3), 249–250.

Maroušek J., Maroušková A., Periakaruppan R., Gokul G.M., Anbukumaran A., Bohatá A., Kriz P., Barta J., Cerny P. and Olšan P. (2022). Silica nanoparticles from coir pith synthesized by acidic sol-gel method improve germination economics. *Polymers*. **14**(2), 266.

Patel R., Shroff P.M., Parmar P.M. and Shah S.N. (2021). Effect of varying fertility levels and biofertilizer on growth, yield and economics of fenugreek. *In Biological Forum-An International Journal*, **13**(3a), 210–213.

Rahmani M., Hamel L., Toumi-Benali F., Dif M.M., Moumen F. and Rahmani H. (2018). Determination of antioxidant activity, phenolic quantification of four varieties of fenugreek Trigonella foenum graecum L. seed extract cultured in west Algeria. *Journal of Materials and Environmental Science*, **9**(6), 1656–1661.

Ríos F., Fernández-Arteaga A., Fernández-Serrano M., Jurado E. and Lechuga M. (2018). Silica micro-and nanoparticles reduce the toxicity of surfactant solutions. *Journal of hazardous materials*, **353**, 436–443.

Rovani S., Santos J.J., Corio P. and Fungaro D.A. (2019). An alternative and simple method for the preparation of bare silica nanoparticles using sugarcane waste ash, an abundant and despised residue in the Brazilian industry. *Journal of the Brazilian Chemical Society*. **30**, 1524–1533.

Ruiz AI., de la Rubia M.Á., Moragues A. and Reyes E. (2024). Influence of nanosilica in the chloride binding capacity of sustainables ground blast furnace slag and metakaolin. Boletín de la Sociedad Española de Cerámica y Vidrio, **63**(3), 187–202.

Saritha G.N.G., Anju T. and Kumar A. (2022). Nanotechnology-Big impact: How nanotechnology is changing the future of agriculture?. *Journal of Agriculture and Food Research*. **2022**, 10, 100457.

Sembada A.A. and Faizal A. (2022). Protein and lipid composition of duckweeds (Landoltia punctata and Wolffia arrhiza) grown in a controlled cultivation system, *Asian Journal of Plant Sciences*. **21**(4), 637–642.

Sembada A.A. and Lenggoro I.W. (2023). Comparative analysis of germination performance from several species of seeds under influence of silica nanoparticles. *In IOP Conference Series: Earth and Environmental Science, IOP Publishing*. **1271**(1), 012085.

Siddiqui M.H. and Al-Whaibi M.H. (2014). Role of nano-SiO_2 in germination of tomato (Lycopersicum esculentum seeds Mill.). *Saudi journal of biological sciences*. **21**(1), 13–17.

Singh R.P., Handa R., Manchanda G. (2021). Nanoparticles in sustainable agriculture: An emerging opportunity. *Journal of controlled release*, **329**, 1234–1248.

Suriyaprabha R., Karunakaran G., Yuvakkumar R., Rajendran V. and Kannan N. (2014). Foliar application of silica nanoparticles on the phytochemical responses of maize (Zea mays L.) and its toxicological behavior. *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry*, **44**(8), 1128–1131.

Tabassum H. and Ahmad I.Z. (2021). Trigonella foenum-graecum and its bioactive compounds having potential anti-diabetic activity. *Fenugreek: Biology and Applications,* **2021**, 447–480.

Tripathi D.K., Singh S., Singh V.P., Prasad S.M., Dubey N.K. and Chauhan D.K. (2017). Silicon nanoparticles more effectively alleviated UV-B stress than silicon in wheat (Triticum aestivum) seedlings. *Plant physiology and biochemistry*, **110**, 70–81.

Wang P., Wang X., Yu S., Zou Y., Wang J., Chen Z. and Wang X. (2016). Silica coated Fe3O4 magnetic nanospheres for high removal of organic pollutants from wastewater. *Chemical Engineering Journal*, **306**, 280–288.

Xiong H., Hua L., Reyna-Llorens I., Shi Y., Chen K.M., Smirnoff N., Kromdijk J. and Hibberd J.M. (2021). Photosynthesis-independent production of reactive oxygen species in the rice bundle sheath during high light is mediated by NADPH oxidase. *Proceedings of the National Academy of Sciences*, **118**(25), e2022702118.

Yadav V.K. and Fulekar M.H. (2019). Green synthesis and characterization of amorphous silica nanoparticles from fly ash. *Materials Today: Proceedings*. **18**, 4351–4359.