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Magnesium Sulfide Nanoparticles of *Hordeum vulgare*: Green Synthesis and their Nano-nutrient Impact on Seed Priming Effect, Germination, Root and Shoot Length of *Brassica nigra* and *Trigonella foenum-graecum*

M. Kezia Elizabeth[1], Randhi Uma Devi[2,*], Makam Parameshwar[3] and A. Ratnamala[2]

[1]School of Science, GITAM Deemed (To Be) University, Visakhapatnam-502329, India
[2]School of Science, GITAM Deemed (To Be) University, Hyderabad-530045, India
[3]Department of Chemistry, School of Applied and Life Sciences, Uttaranchal University, Premnagar, Dehradun-248007, India

*Corresponding author: E-mail: urandhi@gitam.edu

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This work describes the green synthesis of magnesium sulfide nanoparticles (MgS NPs) using an extract from *Hordeum vulgare* leaves and characterized by X-ray diffraction (XRD), UV-visible spectroscopy and scanning electron microscopy (SEM) techniques. The MgS NPs generated were spherical and had a high level of purity. They had a band gap of 2.0 eV, a uniform distribution and an average crystal size of 14 nm. The synthesized MgS nanoparticles exhibited several geometrical morphologies, including spherical, rod-shaped and bean-shaped structures. The nanoparticles exhibited an average size of 5 nm with a band gap of precisely 4.85 eV. The efficacy of MgS NPs on *Brassica nigra* and *Trigonella foenum-graecum* for seed priming, germination rate and time, root length and shoot length has also been assessed using different doses. The optimal germination occurs at concentrations of 15 mg/100 mL and 20 mg/100 mL, while the germination is impeded when the concentration exceed 30 mg/100 mL. The MgS NPs exhibit a diminutive size and high reactivity, which allows them to augment the water absorption and nutrient control capacities of seeds. Consequently, this promotes the germination process and plant growth by decreasing the average duration of germination. Seeds of *Brassica nigra* and *Trigonella foenum-graecum* that had been subjected to treatment with MgS NPs had enhanced average root and shoot lengths, as well as accelerated germination. This study suggests several possibilities for using environmental friendly nanotechnology to improve agricultural practices.

Keywords: Green synthesis, Magnesium sulphide, *Brassica nigra*, *Trigonella foenum-graecum*, Seed priming, Germination rate.

INTRODUCTION

Nanotechnology is a fascinating and ever-growing field that enables the fabrication of extremely small materials [1,2]. Applications of nanotechnology have vastly improved impact on electronics [3], therapeutics [4], food [5,6], sustainable agriculture [7], chemical and pharmaceutical industries [8,9] and environmental health [10]. Top-down or bottom-up methods can regulate nanomaterial dimensions, morphologies and properties. Advanced materials science and technology studies is stressing 'green synthesis' methodologies [11] to monitor, purify and restore nanomaterials to improve their ecological sustainability [12,13]. Green metallic nanoparticles are made from bacteria, fungi and algae [14,15] but for larger quantities preparation, using plant extracts is easier than utilizing microbes. Developing reliable, durable and environmental friendly synthetic procedures is essential for "green synthesis," which eliminates unwanted or harmful byproducts. To achieve this, appropriate solvent systems and natural resources are needed [16,17].

Conventional agriculture relies on pesticides and fertilizers, degrading the environment and polluting natural resources. The global population is anticipated to exceed 9-10 billion by 2050, requiring a 25-70% increase in the food production [18]. Thus, modern agricultural technology is essential for sustainability and production. Nanofertilizers and nanopesticides can revolutionize agriculture by cleaning up polluted water and soil and reducing fertilizer and pesticide use [19]. Plant establishment in agriculture requires good seed germination and healthy seedlings. A quiescent, fully grown seed imbibes water and the embryonic axis, usually the radicle grows into a root and shoot. Nano-priming improves seed viability in poor environments [20] and can preserve seeds, boost germination, synchronize germination, accelerate plant growth, protect crops from abiotic and biotic stress and reduce fertilizer and insecticide use [21,22].

Plant growth and development require magnesium, which is essential to plant for chlorophyll and chloroplasts. In contrast, magnesium is essential for plant enzyme activation. Most kinases and phosphorylases require Mg^{2+} to activate. ATP or ADP hydrolysis by magnesium (Mg^{2+}) produces phosphoric acid and energy. It also boosts phosphorylation and ATP production [23,24]. Conversely, sulfur-rich soils are known for their agricultural potential. Certain fungal species cannot grow in sulfur-rich soils [25,26]. *Hordeum vulgare* or barley, is a cereal grain produced in temperate climates worldwide. The global use of *Hordeum vulgare* crop is 70% animal feed and 30% alcohol and food additives. The study of *Hordeum vulgare* hay for algae control in ponds and other freshwater sources is fascinating [27]. *Hordeum vulgare* biofuel research has begun, although with restricted scope. Magnesium sulfide is the most rare macronutrient in the agricultural sector and it act as enzyme cofactor and tissue stabilizer [28]. The study examined the green synthesis of magnesium sulfide nano-particles (MgS NPs) using an extract from *Hordeum vulgare* leaves, characterization studies and its impact on *Brassica nigra* and *Trigonella foenum-graecum* towards the seed priming, germination rate and time, root length and shoot length.

EXPERIMENTAL

The chemicals/reagents used in this study were sodium sulfide hydrate ($Na_2S{\cdot}H_2O$) and magnesium nitrate hexahydrate [$Mg(NO_3)_2{\cdot}6H_2O$], purchased from Merck Chemicals Ltd., India. *Hordeum vulgare*, *Brassica nigra* and *Trigonella foenum-graecum* seeds were purchased from Seed Preservation Laboratory, Hyderabad, India. *Hordeum vulgare* leaves were obtained from seeds sown for 20 to 25 days at Gandhi Institute of Technology and Management (GITAM), Hyderabad, India.

***Hordeum vulgare* leaf extract:** Seeds of *Hordeum vulgare* were planted in GITAM's Hyderabad campus and allowed to grow for 25 days. These leaves were collected and allowed to air-dry at room temperature for 24 h while being covered. After that the leaves were reduced to a powder form. A solution of plant extracted with 10% (w/v) at 80 ºC under vigorous stirring for 1.5 h. The components were centrifuged for 5 min at 3,000 rpm and the supernatant fluid was collected and stored at 40 ºC for further use as a key raw material for synthesizing the phytocapped MgS NPs.

Synthesis of MgS NPs: Aqueous solutions of $Mg(NO_3)_2{\cdot}6H_2O$ (50 mL, 1 M) and $Na_2S{\cdot}H_2O$ (50 mL, 1 M) were heated at 40 ºC for 0.5 h separately. These two solutions were mixed slowly for 3 h with continuous stirring for 3 h results in the formation of MgS NPs as precipitate. After filtration, the solid portion was washed with distilled water thoroughly and then dried in an oven at 80 ºC for 5 h. The dry sample obtained was ground into a powder with a mortar and pestle and it yielded a fine MgS NPs powder.

Green synthesis of MgS NPs from *Hordeum vulgare* leaves: The extract of *H. vulgare* leaves was separately added to $Mg(NO_3)_2{\cdot}6H_2O$ (50 mL, 1 M) and $Na_2S{\cdot}H_2O$ (50 mL, 1 M) in equal parts at 40 ºC with constant stirring for 3 h. Then the above two solutions were mixed dropwise to obtain the precipitation of *H. vulgare* leaf extract capped MgS NPs. The capped MgS NPs were washed thoroughly with ethanol followed by distilled water before drying at 80 ºC for 5 h in a hot air oven. After being dried, the sample was then crushed using a mortar and pestle to form a fine powder.

Germination tests for seed priming: *Brassica nigra* (mustard) and *Trigonella foenum-graecum* (Fenugreek) seeds were utilized to test the impact of *H. vulgare* (Barley) capped MgS NPs on the germination rate, shoot and root length. *B. nigra* and *T. foenum-graecum* seeds were immersed in distilled water with 20 mg of MgS NPs for 3, 6 and 9 h. The objective was to assess the shorter soaking times to enhance the germination and longer durations to improve the root length. To order to investigate the effects of different concentrations of *H. vulgare* capped MgS NPs, nanoparticles were distributed in distilled water at 5, 10, 15 and 20 mg. The seed germination rate was calculated using eqn. 1:

$$\text{MGT} = \frac{\Sigma(n \times d_i)}{N} \tag{1}$$

where n is the number of newly germinated seeds on an i^{th} day and, d_i is the number of days, N denotes the number of seeds that have just started to sprout, whreas the germinability is assessed using eqn. 2:

$$\text{GE }(\%) = \frac{C}{E} \times 100 \tag{2}$$

[*Note: observations of germination were carried out on the third and seventh days]; where GE = germinability, C = number of seeds that germinated by 3rd day and E = total number of seeds.

Mean germination time (MGT) was calculated by using the equation:

$$\text{MGT} = \frac{\Sigma(n \times d)}{N} \tag{3}$$

where n = daily seed germination rate, d = time in days since the test began and N = total number of seeds that sprouted by the end of test.

RESULTS AND DISCUSSION

UV-visible studies: The UV-visible absorption spectra of MgS NPs obtained from *Hordeum vulgare* leaf extract were analyzed using a UV-1800 apparatus operating at room temperature. The study revealed that MgS NPs exhibited absorption spectra ranging from 200 to 800 nm (Fig. 1). The green synthesized MgS NPs have a linear nature, rendering them suitable as direct band materials. The MgS NPs possess a highly desirable semiconducting band gap of 2.0 electron volts (eV). The presence of a distinct band gap emission in the metal sulfide nanoparticles indicates their exceptional level of crystallinity. The presence of nanoparticle materials is evidenced by the blue

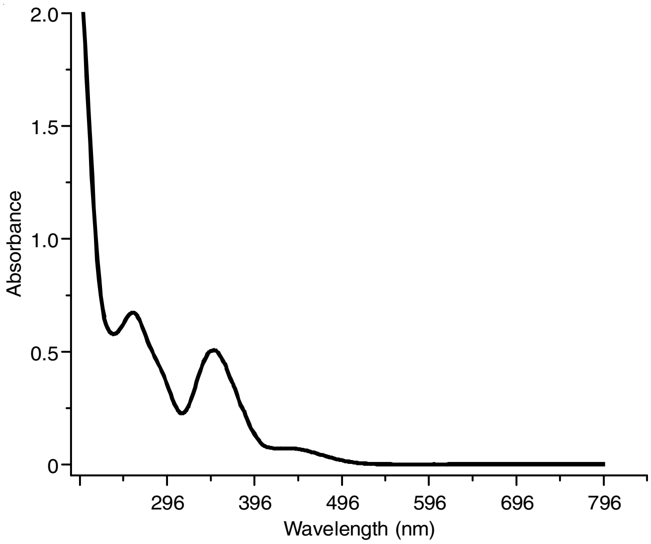

Fig. 1. UV-vis spectra of *Hordeum vulgare* capped MgS NPs

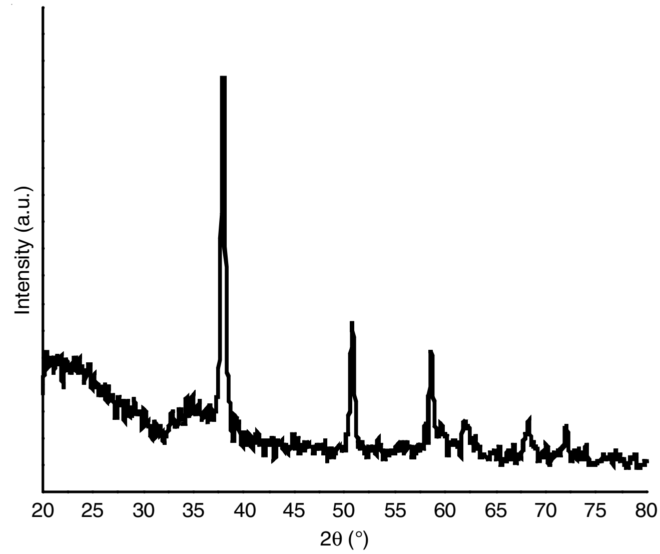

Fig. 2. XRD pattern of *Hordeum vulgare* capped MgS NPs

shift observed in the band gap, which arises from the quantum confinement effect and the entrapment of excitons. The findings are consistent with the previous studies [29]. The greater band gap signifies that nanoparticles are absorbing a bigger amount of light. Due to their higher capacity for absorbing visible light, nanoparticles have the potential to accelerate the process of germination.

XRD studies: The FX Gieger Series RAD-B equipment from Rigaku (Japan) for X-ray diffraction analysis. The XRD patterns of MgS NPs (Fig. 2) obtained from *H. vulgare* leaves extract displayed diffraction peaks at 35.04º, 50.10º and 60.20º, which corresponded to the different crystal planes. The positions of these peaks exhibit a strong correlation with the literature that has been previously reported [30]. The XRD results indicate a spherical structure composed of many crystals with a monoclinic arrangement.

The crystal size was determined using Debye-Scherrer's (eqn. 4):

$$D = \frac{0.98 \times \lambda}{\beta \cos\theta} \tag{4}$$

The size of the crystallite is represented by D, β respresents the broadening of the diffraction lines and λ = wavelength of X-ray having a specific value of 1.5406 Å. An average size of 14 nm were determined for the green synthesized MgS NPs, aligning with the data presented in the reference [31].

SEM studies: The SEM-JSM-5910 was used to analyze the microsurface structures and morphology of the synthesized MgS NPs at different magnifications. As shown in Fig. 3a, the SEM images revealed that the majority of MgS NPs transform into the spherical clusters when they assembled [32]. The SEM images revealed the particles with a size as tiny as 10 nm, suggesting a size range between 10 and 50 nm. The SEM image in Fig. 3b revealed the presence of MgS NPs in many forms, such as spherical, rod-like, bean-like and other irregular shapes.

Applications of *Hordeum vulgare* capped MgS NPs

Effect on seed priming: Germination is one of the most critical phases in guaranteeing moisture affirmation and if done correctly, it can also help speed up the process of plant growth, which can be beneficial. Due to their extremely small size, nano-

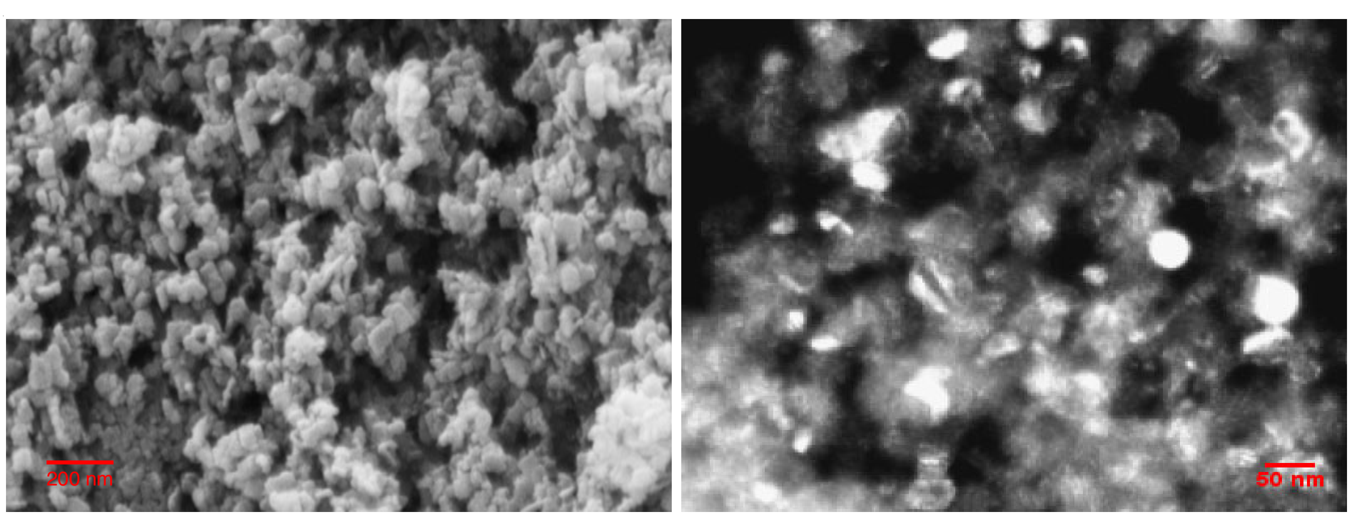

Fig. 3. SEM images of *Hordeum vulgare* capped MgS NPs (a) spherical clusters (b)in different forms

particles may allow water and other nutrients to penetrate seeds without harming their core composition. Plants can take up more nutrients with less runoff when using nanoparticles as fertilizer [33]. This is because nanoparticles have a greater release rate and a larger surface area than larger particles. As a result, the plant could be able to make better use of the nutrients it receives.

Effect of germination rate: Germination and seed vigour are the reliable indicators of a crop's performance in the field. The seeds should take two days to take up moisture and four days to form a root and a leaf, although the germination rate might vary depending on the variety [34]. A different percentage of germination occurs when MgS NPs suspensions were used instead of the control treatment. The effects of *H. vulgare* leaf extract on the germination rate of *B. nigra* and *T. foenum-graecum* seeds, as well as the control treatment, are depicted in Figs. 4 and 5, respectively. The germination rate is the proportion of seeds that germinate to the total number of seeds planted. The maximum germination rates were obtained with concentrations of 15 mg/100 mL and 20 mg/100 mL. The increased germinability at 30 mg/100 mL does not translate to an increased germination rate. It shows that a larger dose at the beginning of germination can halt the germination process. High

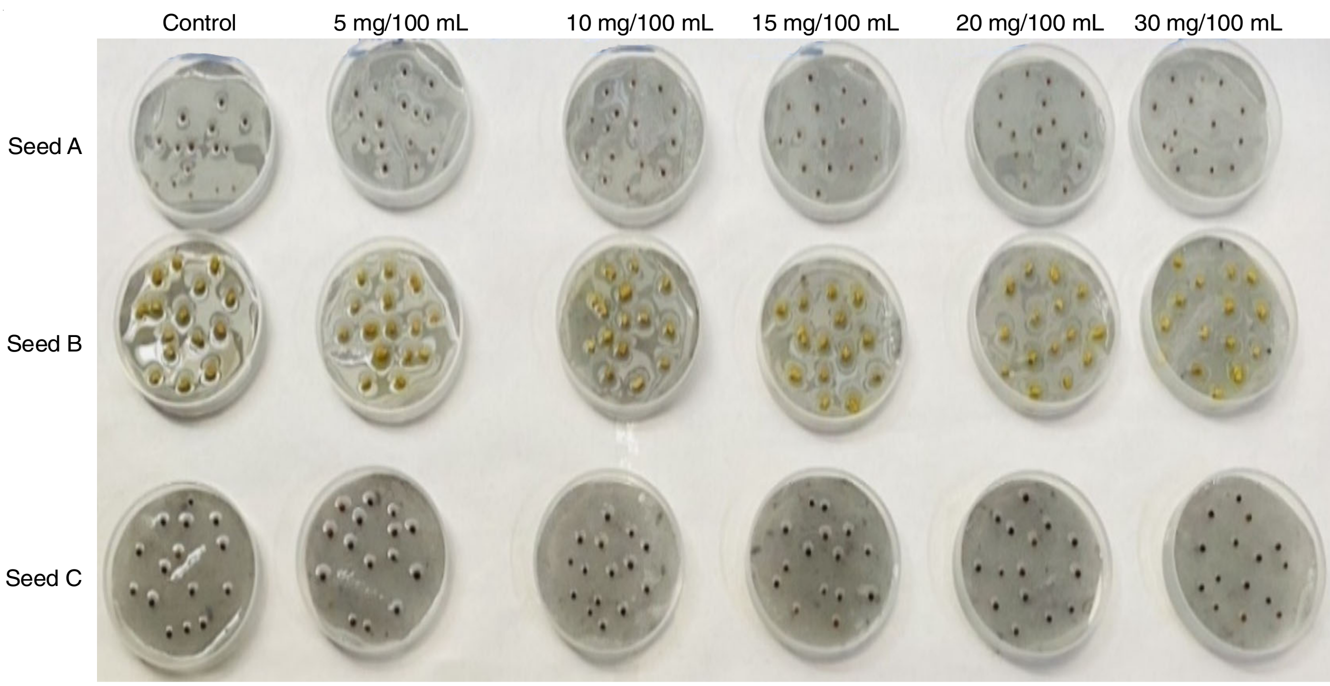

Fig. 4. Seed germination studies of *Hordeum vulgare* capped MgS NPs. Seed (a) *Trigonella foenum-graecum* (b) *Brassica nigra*(c) control

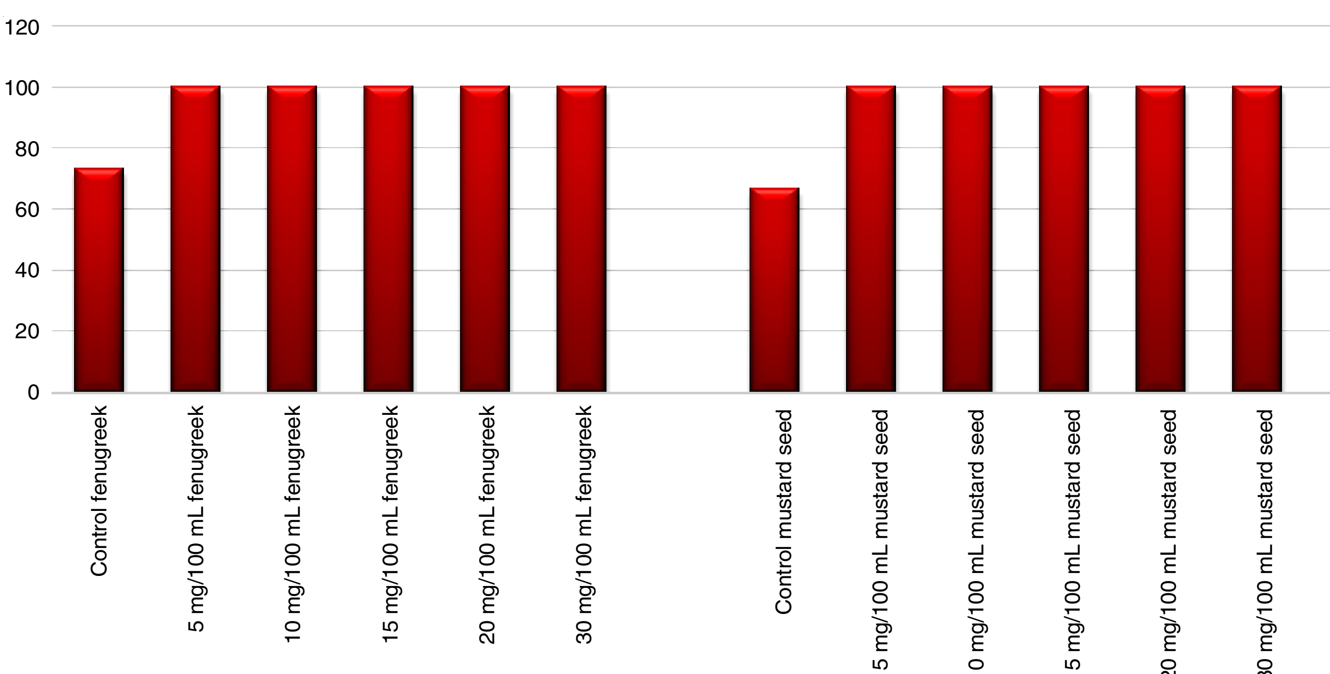

Fig. 5. % Germination rate of *Hordeum vulgare* capped MgS NPs

germination rates can be achieved with low doses of nanoparticle suspension (5 and 10 mg/100 mL) for *T. foenum-graecum* seeds, but for *B. nigra* seeds, much higher concentrations are required. The germination rate is highest for double steric and lowest for single steric [35]. MgS NPs were shown to have a high germination rate (in percentage terms) for both types of seeds. At 20 mg/100 mL, germination of *T. foenum-graecum* seeds was 100%, while at 15 mg/100 mL, germination of *B. nigra* seeds was 86%. The germination rates of both plants are reduced by 30 mg/100 mL. In every case, the high concentration suspension of 30 mg/100 mL nanoparticles had a much higher germination rate than the control treatment. The germination rate was significantly reduced in all cases compared to the nanoparticle dispersion at a 30 mg/100 mL dosage.

Effect on root length: The root length of *B. nigra* and *T. foenum-graecum* seeds increased when they grown in a suspension of MgS NPs in comparison to control seeds, except for 30 mg/100 mL concentration of nanoparticles for *T. foenum-graecum* seeds, which showed a significantly reduced root length. At the dosages of 10 mg/100 mL, 15 mg/100 mL and 20 mg/100 mL, the root length of both varieties of seeds rises. The best concentrations for maximum size were 10 mg/100 mL for *B. nigra* seeds and 15 mg/100 mL for *T. foenum-graecum*. Here, the root length is significantly reduced with greater concentrated dosages of nanoparticles because nanoparticles at higher concentrations are more reactive, toxic and have the potential to inhibit and suppress the development process, which can result in the shorter roots [36].

The ability of plant to absorb water and light has been enhanced by the MgS NPs, leading to an increase in root length. In order to attain healthy and long roots, it requires a suspension of nanoparticles in a solution appropriate concentration. Thus, the maximum shoot and root lengths of 2.0 cm and 3.1 cm for *T. foenum-graecum* and *B. nigra* seeds was 0.3 cm and 0.9 cm was attained while using the green synthesized MgS NPs. These values are significantly greater than those observed for seeds that were given a control treatment. MgS NP solutions of 10 and 15 mg/100 mL were shown to produce the maximum root length in *B. nigra* and *T. foenum-graecum* seeds, respectively. However, at higher doses (20 and 30 mg/100 mL), there is a noticeable reduction in the root length.

Effect on shoot length: Shoot length is more significant in all concentrations compared to the control treatment, but the effects vary widely (Figs. 6 and 7). In a 7 day study, *T. foenum-graecum* grew to a maximum of 3.5 cm in length when grown in a suspension of MgS NPs, but only 2.0 cm and 1.9 cm when grown in solutions of 20 mg/100 mL and 30 mg/100 mL, respectively. At 15 mg/100 mL, the maximum length of *B. nigra* seeds was 2.5 cm; at lower doses, the seeds were shorter. Therefore, it is deduced that a 15 mg/100 mL of MgS NPs is optimal for producing the longer shoots. This analysis confirms that MgS NPs accelerated the rate of germination.

Mean germination time analysis: The mean germination time (MGT) for *T. foenum-graecum* at 15 and 20 mg/100 mL is reduced to 1.4 days, however, it is increased to 1.9 days for control seeds. The MGT for *B. nigra* is reduced to 1.3 days, whereas it is increased to 1.6 days for control. The results of MGT using 15 mg/500 mL of MgS NPs were 1.2 and 1.3 for *B. nigra* and *T. foenum-graecum* seeds, respectively. The MGT concentrations of *B. nigra* and *T. foenum-graecum* seeds are presented in Table-1. MGT can be speed up to 1.2 days for *T. foenum-graecum* seeds and 1.1 days for *B. nigra* seeds when low amounts of MgS NPs are present, such as 10 mg/100 mL. Low concentrations lowered the minimum germinability threshold (MGT) and the number of germinating seeds increased regardless of the rate at which they were growing [37,38]. At

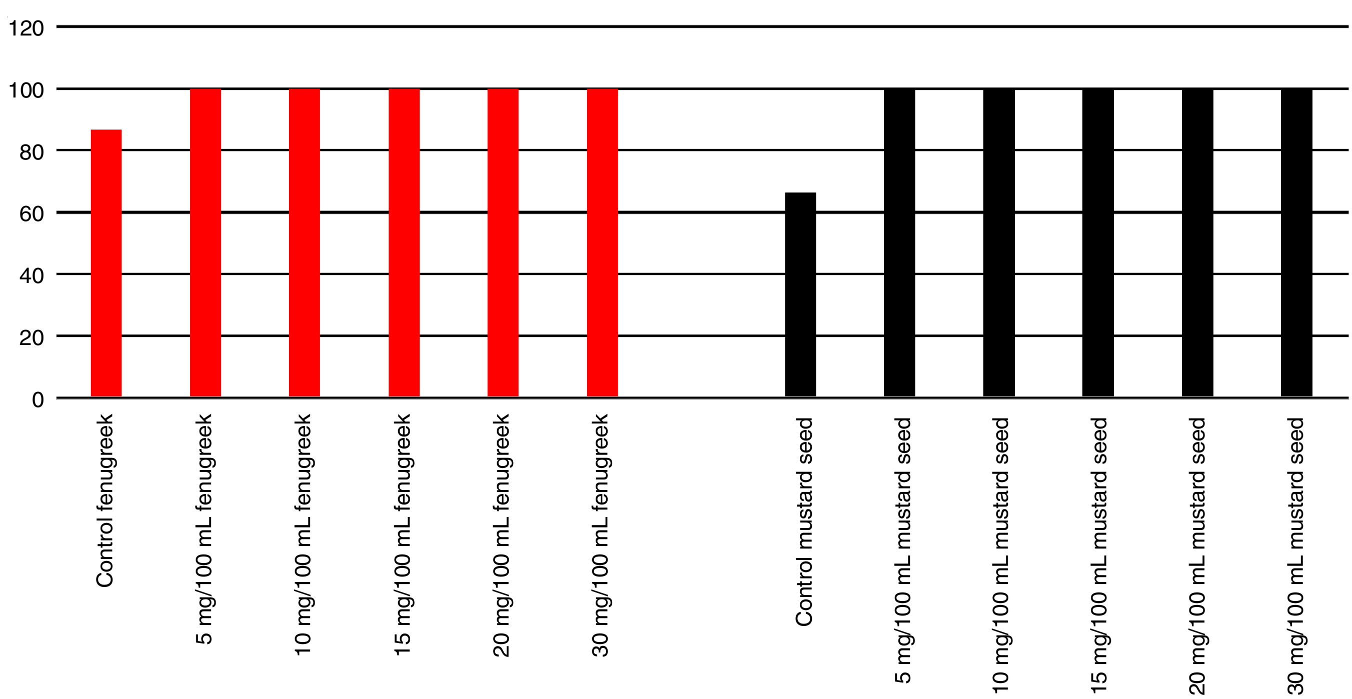

Fig. 6. Effect of *Hordeum vulgare* capped MgS NPs on % of germination rate in *Trigonella foenum-graecum* and *Brassica nigra* and control

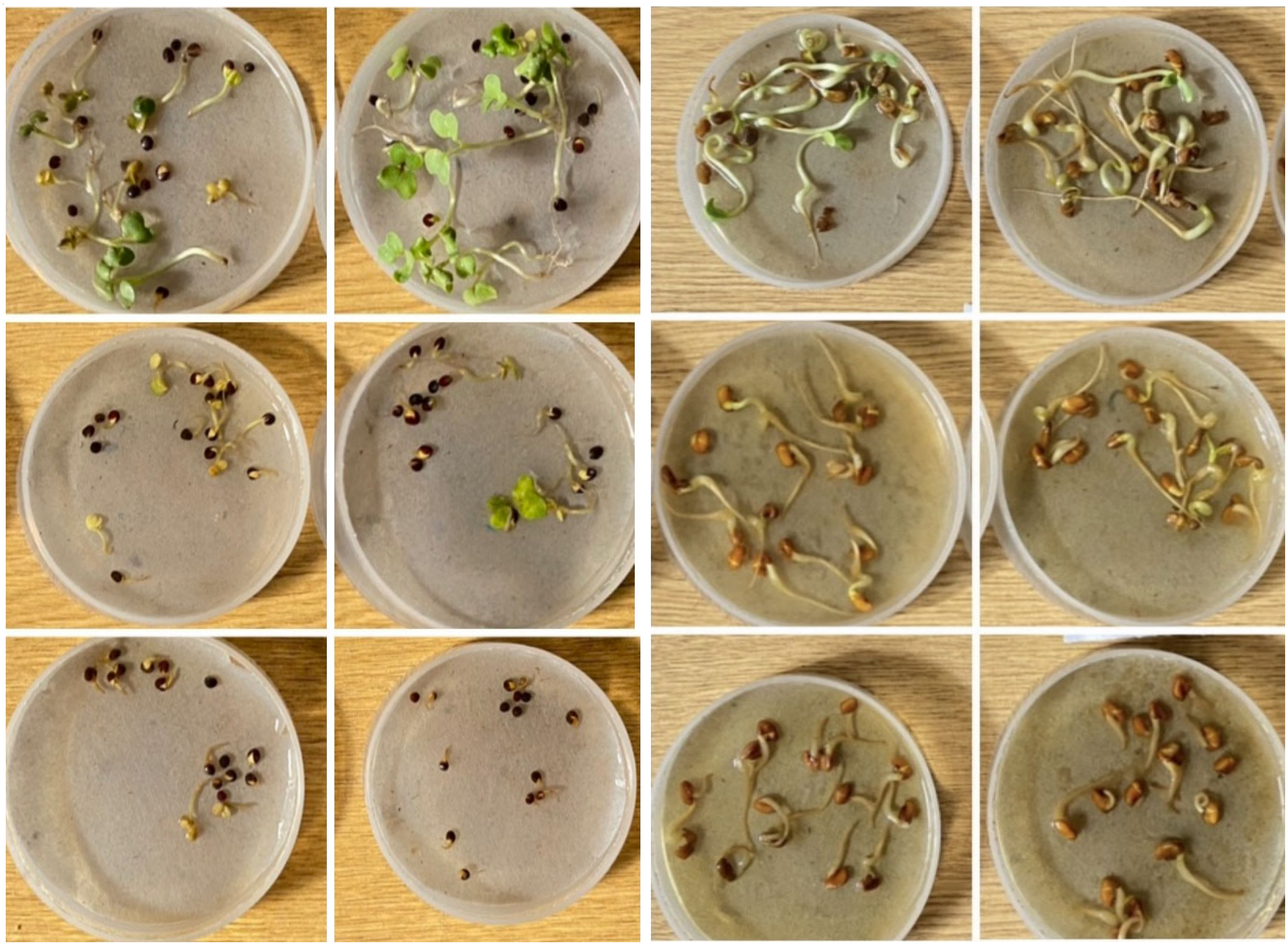

Fig. 7. Shoot and root developed in *Hordeum vulgare* capped MgS NPs treated seeds

TABLE-1
GERMINABILITY AND GERMINATION RATE

	Trigonella foenum-graecum		*Brassica nigra*	
	% of Germinability (3^{rd} day)	% of Germination rate (7^{th} day)	% of Germinability (3^{rd} day)	% of Germination rate (7^{th} day)
Control	73.3	100	66.6	100
5 mg/100 mL	86.6	100	60	100
10 mg/100 mL	86.6	100	66.6	100
15 mg/100 mL	93.3	100	86.6	100
20 mg/100 mL	86.6	100	80	100
30 mg/100 mL	73.3	100	73.3	100

high concentrations, for example, 30 mg/100 mL, the germination time recorded was substantial, nearly 2 days for *T. foenum-graecum* seeds; utilizing only a sufficient number of nanoparticles can assist reduce this time. The germination and growth rates of seeds are influenced by the duration of their immersion in a solution containing metal sulfide nanoparticles. Germination tests were conducted after 3, 6 and 9 h of immersion. There is no effect on root or shoot length, although germination and viability are greatly improved by a 3 h soak. The germination rate and viability of seeds soaked for 9 h were reduced, but the resulting root growth was far more impressive than that of seeds soaked for only 3 or 6 h. While soaking for 6 h does not appear to have much of an effect on any germination test, soaking for 9 h decreases the germination rate but increases root length. Soaking for 3 h promotes germination and 9 h promotes root growth. When compared to suspension, the MgS NPs isolated from *H. vulgare* have excellent germinability. However, the germination rate is low regardless of the length of time the seeds were soaked. A 3 h soak lengthens both the roots and the shoots. Therefore, the optimal concentrations of MgS NPs must be used to improve germination rates and agricultural output.

Conclusion

The leaf extract of *Hordeum vulgare* was subjected to chemical precipitation, leading to the formation of *H. vulgare* capped MgS NPs. These nanoparticles were analyzed using UV-visible spectroscopy, XRD and SEM techniques. The spherical *H. vulgare* capped MgS NPs produced exhibited exceptional purity, with a band gap of 2.0 eV, a homogeneous distribution and an average crystalline size of 14 nm. The MgS NPs produced were found in many geometrical forms, such as spherical, rod-shaped and bean-shaped structures. Germi-nation is the most favourable at concentrations of 15 mg/100 mL and 20 mg/ 100 mL, but it is hindered when the concentration exceeds 30 mg/100 mL. *H. vulgare* capped MgS NPs possess a small size and high reactivity, enabling them to enhance the water absorption and nutrient regulation capabilities of seeds. This, in turn, facilitated both the germination process and the growth of plants and a reduction in the average germination period. The germination and growth rate were influenced by the characteristics and dimensions of nanoparticles. Seeds of *Brassica nigra* and *Trigonella foenum-graecum* that had undergone treatment with MgS NPs exhibited greater average root and shoot lengths, as well as faster germination. The findings of this study indicate several compelling prospects for exploring the utilization of eco-friendly nanotechnology in enhancing agricultural methods.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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