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GREEN SYNTHESIS OF FeS NANOPARTICLES FROM *Hordeum vulgare*LEAF EXTRACT AND EVALUATION OF ITS IMPACT ON SEED PRIMING AND GERMINATION

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ABSTRACT

In this study, we used *Hordeum vulgare* leaf extract as a sustainable environmental approach for producing FeS nanoparticles (FeS NPs). The synthesized FeS NPs were examined using the SEM, UV-Vis, and XRD powder diffraction techniques. XRD tests validated the structure of FeS nanoparticles. These nanoparticles had an average 14 nm crystal size and possessed a 2.0 eV band gap with an even distribution. A further study was conducted to investigate *Hordeum vulgare* capped FeS nanoparticle's impact on seed priming and germination characteristics. The seed priming effect on the germination of *Trigonella foenum-graecum*and *Brassica nigra* seedswas examined using various concentrations of phyto-capped FeS NPs. The Germination rate occurs optimally at doses of 15mg and 20mg, but the germination rate is retarded at 30mg of FeS NPs in both the seed types. FeS NPs are tiny and highly reactive, allowing them to improve seed water uptake and nutrient management. As a result, this enhances germination and plant growth by shortening the average germination period. Therefore, FeS NPs developed using the green method if used in optimal concentration exhibit significant promise for the advancement of agricultural practices.

Keywords: Green Synthesis, FeS NPs, Germination, Shoot, and Root Length.

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INTRODUCTION

Metal-based nanoparticle production using green technologies has gained interest in the past decade. Green synthesis produces biocompatible, eco-friendly nanomaterials with non-toxic ingredients and less trash than physicochemical methods.[1] Green nanoparticle synthesis reduces metal precursors to nanoparticles using phytochemical-rich plant extracts like polyphenols and flavonoids. Green synthesis of metallic NPs from leaves, stems, roots, and seeds is inexpensive, simple, and reproducible. Plants naturally produce stable metal NPs. Thus, green-synthesized NPs of plant extracts are better for quick, large-scale synthesis than other green synthesis methods that need particular conditions. Herb, fruit, leaf, and stem antioxidants boost NP capacity. Figure-1 shows that employing plant-based phytochemicals to synthesize NPs combines natural/plant sciences and nanotechnology to offer safe, environmentally beneficial, justified, and economically practical green technologies.

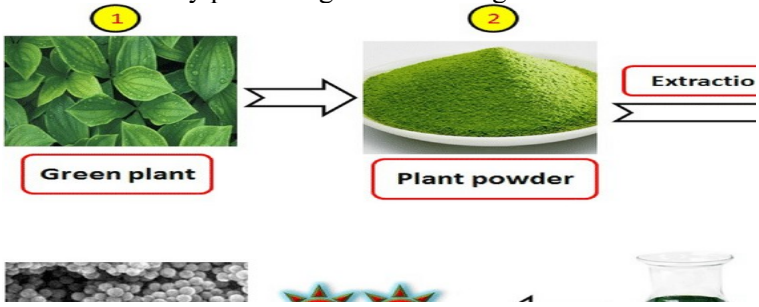

Fig.-1: Plant-based Green Nanotechnology

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Chemical fertilizers, pesticides, climate change, and resource depletion threaten agriculture. Fertilizers and pesticides pollute the natural resources in traditional agriculture. Food production must increase 25-70% by 2050 to feed 9-10 billion.[2] Therefore new agricultural technology is needed to boost output and sustainability. A tech-driven agricultural revolution may result from green nanotechnology. Plant growth and reproduction require iron. Chlorosis in plants can also be reversed by providing iron. Plants need modest amounts of microiron[3]. Studies on nanoscale iron sulfide particles for energy storage, medicine, catalysis, and environmental cleansing are being researched.[4] As nanoscale iron sulfides are becoming more popular but only a few researchers have examined green nanoscale iron sulfide synthesis. Hence in this research work, a novel greener approach is followed for Fes NPs generation. Barley, *Hordeum vulgare*, is a grass grown as a cereal grain. This study synthesizes FeS NPs from barley leaf extract using green technology and evaluates their seed priming effects on *Trigonella foenum-graecum* and *Brassica nigra* seeds germination.

EXPERIMENTAL

Hordeum vulgare (Barley) seedswere grown for 28 days, and their leaves were dried. One gram of this leaves powder in 300 ml distilled water is boiled at 90^{o}C for one hour. In order to separate the components of this leaf extract, a centrifuge set at 3,000 RPM is used for 5 minutes and the collected supernatant is stored at 4^{0} C.

Hordeum Vulgare Capped FeS NPs Synthesis

Synthesized *Hordeum vulgare* leaf extract was combined with $Fe(NO_3)_3$ and Na_2S solutions in equal proportions to form FeS Nanoparticles. An equal ratio of leaf extract and Ferrous nitrate in a beaker is heated at 40 ^{0}C for 50 minutes on a magnetic stirrer. This process requires a 1M solution of $Fe(NO_3)_2$ and a 1M solution of Na_2S. 50 ml of 1M Na_2S was heated separately at 40 ^{0}C for 60 minutes. Then, using a burette, 50 ml of Na_2S (1M) was gently added to the leaf extract combined $Fe(NO_3)_3$ (1M) solution while constantly stirring for 3 hours to investigate the precipitation of Fe NPs. The addition of the Na_2S to the phyto $Fe(NO_3)_2$ solution, resulted in a precipitate of phyto-capped FeS NPs. Using demineralized water as well as ethanol, the precipitate was washed thoroughly before being dried. Barley-capped FeS NPs were created through green synthesis, resulting in a fine powder. The morphology of the obtained sample was characterized using SEM (Scanning Electron Microscopy), UV-Vis, and XRD powder diffraction (X-ray diffraction). Further, barley-capped FeS Nps impacts were examined on the germination of*Trigonella foenum-graecum* (Fenugreek) seeds, and *Brassica nigra*(mustard) seeds.

Germination Tests+

Germination and seed vigor accurately predict the field performance of crops.[5] Seeds should absorb moisture in two days and sprout roots and leaves in four days, but germination rates vary by variety.[6]N5 mg of phyto-capped FeS NPs were dissolved in 100 ml deionized water along with 10 mg of *Hordeum vulgare* capped FeS, 15 mg of *Hordeum vulgare* capped FeS, 20 mg of *Hordeum vulgare* capped FeS, and 30 mg of *Hordeum vulgare* capped FeS concentrations separately for germination studies. Seedlings of *Trigonella foenum-graecum* seeds and *Brassica nigra* seeds were planted on Petri dishes with filter paper at the bottom and applied to the prepared phyto-capped metal sulfide suspensions. Phyto metal sulfide nanoparticle solutions were applied to the seeds in the above-saidorder for identification. 15 seeds of each type of seed are grown in the 120mm glass petri dishes. The prepared concentrations of *Hordeum vulgare-capped* FeS NPs are applied to them. A control was maintained to observe if nanoparticles affect seed germination or not. Third and seventh-day germination observations were used to compute germinability using the following equation:

$$\text{Percentage of Germinability (\% PG)} = GS / TS * 100 \quad (1)$$

Where GS represents the 3rd day germinated seeds, and TS is the total seeds applied.

The rate of seed germination for the selected seeds is calculated by the following eqn.-2.

$$\text{Seed Germination rate (\%)} = TS/SS * 100 \quad (2)$$

In this, TS is the total seeds applied for research in the 120 mm glass petri plates, and SS is the 7th day sprouted seeds. This experiment was performed three times and recorded all of our findings. Calculated the root, and shoot lengths of each Nanoparticle concentration in centimeters after seven days. Daily every reading was recorded, including the number of germinated seeds, throughout the counting procedure. Finally, we used the calculation in Eq.-3 to get the typical germination period.

$$\text{Germination period (MGT)} = \sum(\text{NS} \times \text{DG}) / \text{TS}, \quad (3)$$

Where NS indicates the number of newly germinated seeds number, DG is the days of germination, and TS denotes the total number of seeds.

RESULTS AND DISCUSSIONS

UV-Visible and XRD Studies

Absorption spectroscopy encompasses the visible spectrum, whereas UV-visible spectrophotometry encompasses the adjacent ultraviolet spectrum.[7]The absorption spectra of *Hordeum vulgare-capped* FeS nanoparticles were calculated on a UV-1800 spectrophotometer by graphing an arc that starts from the origin. The absorption spectra of FeS nanoparticles ranged from 200 to 800 nm. The suggested FeS nanoparticles are linear direct bandgap materials.[8]FeS NPs were found to have a high-quality semiconducting band gap of 2.0 eV. High bandgap emission of phyto metal sulfide nanoparticles had a well-defined crystalline structure.[9]The UV absorbance spectra of phyto-capped FeS nanoparticles are shown in Fig.-2(a). The band gap value suggests increased light absorption by nanoparticles, leading to enhanced photocatalytic activity. The increase in band gap value, caused by confining electrons and holes in a small area, provides proof of nanoparticle formation.[10]

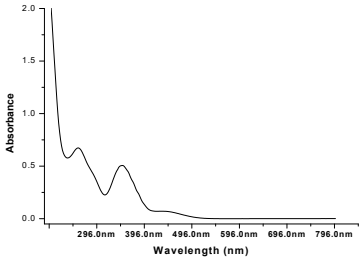


Fig.-2(a): UV Vis of *Hordeum Vulgare* Capped FeS NPs

X-ray diffraction is the preferred method to characterize the synthesized NPs because it interacts with electrons in the atom's inner shell. The photo-capped FeS sample is placed

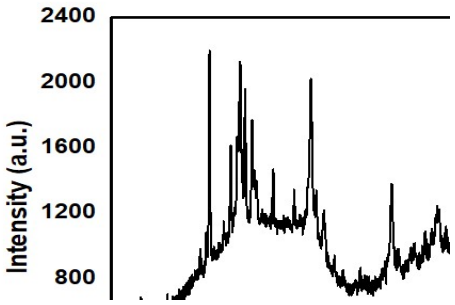


Fig.-2(b): XRD of *Hordeum Vulgare* Capped FeS

on a glass sample holder and accelerated 15 kV in an X-ray generating chamber. Data was analyzed using X-ray diffractometer software and JCPDS cards.[11] Figure-2(b) shows that *Hordeum vulgare* capped FeS NPs had XRD peaks at 30, 35, 40, 50, and 60, corresponding to crystal planes. These peak positions match those in the literature.[12]X-ray diffraction shows a spherical, polycrystalline, monoclinic structure. The graph showed that most particles were in the (30) to (60) planes.

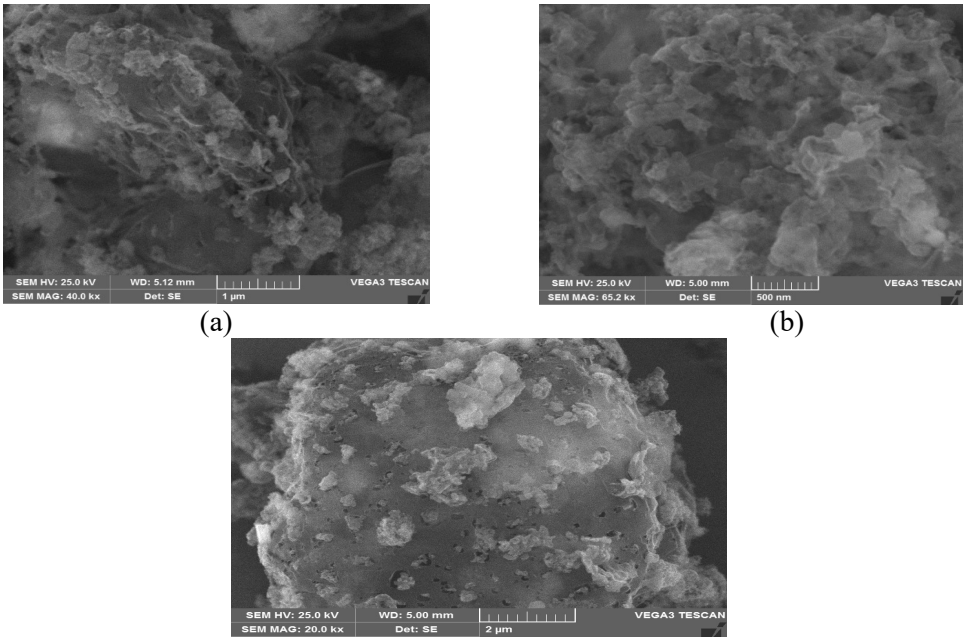
De-bye Scherrer's equation (4) was used to get the typical crystal size.

$$CS = 0.98*\lambda /\beta \cos \theta \qquad (4)$$

CS represents crystallite size, β represents diffraction line widening, and λ, with a value of 1.5406, represents X-ray wavelength. FeS NPs have an average size of 14 nm according to the above formula, which is in line with the data that has been published.[13] The crystalline nature of the generated FeS NPs is demonstrated by the diffraction pattern.

SEM Morphological Study of FeS NPs

Milligram quantities of material can be analyzed by scanning electron microscopy (SEM) to ascertain particle size, shape, and texture. A thin beam of electrons travels across the cleaned and positioned sample in parallel lines in a scanning electron microscope (SEM).[14]Electron signals generated by a sample's interaction can be seen in cathode ray tubes. Using SEM-JSM-5910, the shape and micro-surface structures of the green synthesized FeS NPs were investigated.[15]Variations in the area's properties can be seen in the two-dimensional photographs produced by the SEM. The phyto-capped FeS NP sample was examined at 15 kV using a range of magnifications and scale bar sizes (μm length). The morphology was seen at the following magnifications: (a) 5000 x, 5 μm; (b) 10,000 x, 1 μm; (c) 20,000 x, 1 μm; (d) 2500 x, 10 μm; (e) 60,000 x, 0.2 μm; and (f) 40,000 x, 0.5 μm. The scanning electron micrographs clearly show that almost all of the FeS NPs aggregate into spherical particles as the process progresses. According to the literature[16], SEM images occasionally showed particles as small as 500 nm, suggesting a size range of 10-500 nm. Various morphologies, such as spherical, bean-like, rod-like, and other irregular shapes, were observed in the SEM photographs shown in Fig.-3a, 3b, and 4c. Consistent sizes and distributions of the particles were noted.



(a) (b)

(c)

Fig.-3:(a,b,c) SEM Images of Phyto-capped FeS NPs

Hordeum Vulgare Capped FeS NPs Effects on Germination

Ensuring proper moisture levels is crucial for successful germination and can also accelerate plant growth[17]. Utilizing nanoparticles could potentially enhance plant system efficiency through improved nutrient uptake and utilization. Their minute size enables quicker release and a greater surface area, enabling plants to absorb more nutrients efficiently. The germination rate and growth rate of *Trigonella foenum-graecum* (fenugreek) seeds and *Brassica nigra* (mustard) seeds are studied using different concentrations of *Hordeum vulgare* capped FeS NPs, comparing them to control center seeds.Figures-4(a

and b) shows the effects of phyto-capped FeS NPs on the germination rate of *Trigonella foenum-graecum* and *Brassica nigra* seeds. The results of the germination studies show that the rate of germination differs between the control and *Hordeum vulgare-capped* FeS NPs. The highest germination rates were seen at doses of 15 mg, and 20 mg of phyto FeS NPs. The improved germinability at 30 mg of *Hordeum vulgare* capped FeS NPs does not result in an increased germination rate. It proves that if given at a larger dose before germination begins, it can halt the process. For *Trigonella foenum-graecum* seeds, low doses of phyto-capped FeS NPs suspension (5 mg, and 10 mg) resulted in good germination rates, whereas considerably greater concentrations are required for *Brassica nigra* seeds.

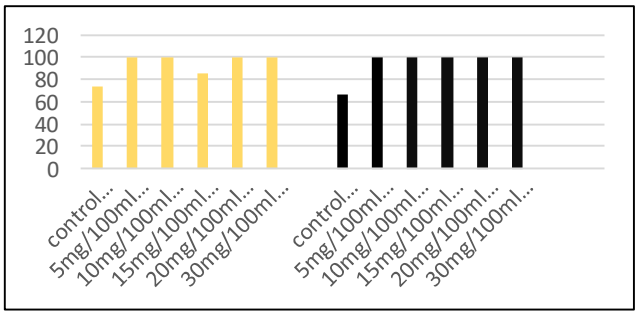


Fig.-4(a): % Germination Rate of Pure FeS NPs

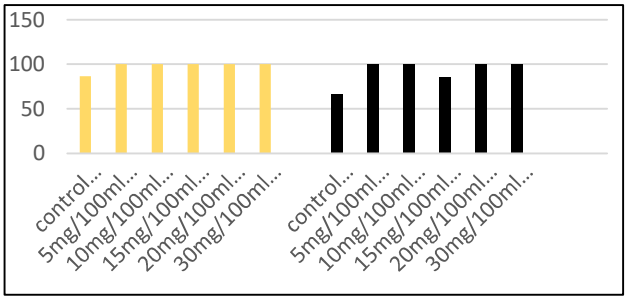


Fig.-4(b): % Germination Rate of FeS NPs of Green Synthesis

Hordeum vulgare-capped FeS NPs resulted from a high % germination rate for the selected germinated seeds. *Trigonella foenum-graecum* seeds germinated 100% at 20 mg *Hordeum vulgare* capped FeS NPs concentration; while *Brassica nigra* seeds germinated 86% at 15 mg *Hordeum vulgare* capped FeS NPs. At 30 mg phyto-capped FeS NPs, the germination has dropped for the studied seeds. The study found that nanoparticle dispersion increases root length even at low concentrations.[18]

Effect of *Hordeum Vulgare* Capped FeS NPson Root Length

Fenugreek and mustard seeds grown with a suspension of *Hordeum vulgare-capped* FeS NPs had longer roots than control seeds, except for the 30 mg of phyto-capped FeS NPs concentration, which had shorter roots. Root length rises at 10 mg, 15 mg, and 20 mg for both seed varieties. The maximal length was achieved at 10 mg for mustard (*Brassica nigra)* seeds and 15 mg for fenugreek (*Trigonella foenum-graecum)*. Greater nanoparticle concentrations limit root length, suggesting a dose-dependent effect. Higher NP concentrations, which are more reactive and poisonous, may slow growth and shorten roots.[19] Root length has grown to metal sulfideNPs due to superior water and light absorption than growth. From this study, it was observed that healthy, long roots need the correct NP suspension concentration.[19] The maximum root length for fenugreek seeds was 2.0 cm and 3.1 cm, and for mustard seeds, it was 0.3 cm and 0.9 cm. Both *Trigonella foenum-graecum* seeds and *Brassica nigra* seeds have the longest roots with 10 mg and 15 mg of phyto-capped FeS NP solutions. However, a 20 mg dose produces short roots. The root length of both seeds decreases at higher dosages (20 mg, 30 mg). This study found that nanoparticle suspensions can promote root length even at low concentrations.

***Hordeum Vulgare* Capped FeS NPs effect on Shoot Length**

Hordeum vulgare-capped FeSnanoparticle suspensions of different concentrations were also observed for shoot length.[20] Shoot length is greater in all *Hordeum vulgare* capped FeSnanoparticle concentrations than in the control seeds, but the effects varied greatly. In a 7-day investigation, fenugreek developed to a maximum length of 2.5 cm in a suspension of phyto-capped FeS NPs. Mustard seeds reached a maximum length of 2.6 cm at 15 mg, lesser doses resulted in shorter seeds. As a result, we may conclude that a concentration of 15 mg of phyto-capped FeS NP is ideal for generating longer shoots. This investigation demonstrates that FeS NPs increased germination.

Analysis of Mean Germination Time (MT)

The eqn.-5 was used to calculate MT.

$$MT = \sum(ge \times nd) / TS \quad (5)$$

Where ge = seeds germinated on each day, nd = a number of days from the beginning of the test, and TS = total germinated seeds at the termination of the experiment.[21,22]Metal sulfide NPs, particularly *Hordeum vulgare* capped FeS NPs, reduce MT for *Trigonella foenum-graecum* at 15mg and 20mg to 1.4 compared to 1.9 for control seeds as well as for *Brassica nigra* to 1.3 compared to 1.6. MT with *Hordeum vulgare* capped FeS Nps at 15mg yielded 1.2 and 1.3 for fenugreek and mustard seeds. Table-1 shows the germination and germinability of selected seeds at different concentrations. It was found that 10 mg *Hordeum vulgare* capped FeS NPs decreases *Trigonella foenum-graecum* seeds MT to 1.2 days and *Brassica nigra* seeds to 1.1 days. The MT for seeds to grow decreased with low concentrations, but those that germinated increased regardless of their growth rate.[23]Soaking seedlings in metal sulfide nanoparticles at different times influences growth and germination.[24]Germination experiments were done after 3, 6, and 9 hours of immersion. Soaking for 3h boosts germinability and germination but does not affect root or shoot length. The seeds soaked for 9h had lower germinability and germination rates than those soaked for 3h and 6h, but they had longer roots. Nine hours of soaking does not improve germination, although root length rises, while six hours does not. Three hours of soaking improves germination and nine hours increases root length. FeS NPs from *Hordeum vulgare* had better germinability than suspension, but germination was low during soaking. Soaking for 3h lengthens roots and shoots in this study. For proper germination, seeds must be soaked for 3 hours and the optimal *Hordeum vulgare* capped FeS NP concentration used to boost agricultural yield.

Table-1: Germinability and Germination Rate for the Selected Seeds on 3rdand 7th Day

Seeds	Germinability (3rd)	Germination Rate (7th)
Trigonella foenum graceum seeds		
Control	73.3	100
5mg/100ml	86.6	100
10mg/100ml	86.6	100
15mg/100ml	93.3	100
20mg/100ml	86.6	100
30mg/100ml	73.3	100
Brassica nigra seeds		
Control	66.6	100
5mg/100ml	60.0	100
10mg/100ml	66.6	100
15mg/100ml	86.6	100
20mg/100ml	80.0	100
30mg/100ml	73.3	100

CONCLUSION

Fes Nanoparticles from *Hordeum vulgare* improved germination and modulated plant psychological responses to fertilization and other growth cues. *Hordeum vulgare* leaves extract capped FeS NPs were characterized for SEM, UV-vis, and XRD powder diffraction. The crystalizable FeS NPs generated had a

2.0 eV band gap with a homogenous distribution of around 500 nm size crystalline nanoparticles, indicating good purity. Using metal sulfide nanoparticles resulted in decreased germination time. Germination capability of *Trigonella foenum-graecum* (fenugreek) seeds and *Brassica nigra* (mustard) seeds are most effective at 15mg and 20mg phyto-capped FeS Nanoparticle dosages, and the germination is hindered at 30mg phyto-capped FeS Nanoparticle dosage. These results proved that phyto metal sulfide nanoparticles are tiny and reactive enough to improve water absorption and regulate nutrient supply to seeds, thus aiding germination and plant growth. This paper suggests numerous promising uses for eco-friendly green nanotechnology in agriculture.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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