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Original Research Article

Synthesis of Green Iron Nanoparticles Using Henna Plant Extract for Seed Germination and Vegetative Growth of *Trigonella foenum-graecumL*

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Abstract: This paper presents the green synthesis of iron nanoparticles (FeNPs). Iron sulfate served as the substrate and henna extract as the reducing agent in the synthesis of FeNPs. FeNPs were analyzed using a variety of techniques, including UV-visible spectroscopy, nano zeta-sizer analysis, and Fourier transform infrared spectroscopy (FTIR). By using UV-visible spectroscopy, the electron transition band of iron oxide, or the peak at 291 nm, was found to be the source of the FeNPs. The characteristic bands of iron oxide were detected at 790 by FTIR. These bands are associated with the stretching vibration of Fe-O at 1022 and 1516 cm-1. to determine how applying different combinations of nano-fertilizers affects the growth and yield of fenugreek. The experiment consisted of nine treatments. The results demonstrated that T8 performed better than the other treatments in terms of all elements of vegetative development and yield contents. The T8 yields plant height of 43.12 cm, fresh weight of 30.41g, dry weight of 2.44g, seed weight of 5.09g, and pod weight of 7.39g with an increase rate of (55.56, 82.97, 162.36, 105.24, and 122.59)%.

Keywords: Iron nanoparticles, FTIR, UV-visible.

INTRODUCTION

The foundation of green nanotechnology is the use of environmentally friendly nanomaterials that are safe for human health or nanoproducts made to address issues with the environment and the medical system [1-3]. Because of its commercial uses across numerous industries, nanomaterials have garnered increasing attention in recent years [4]. Because of their special qualities, which include good stability in ambient circumstances and biocompatibility with both the human body and the environment, iron oxide-based nanomaterials have become more widely employed [5].

The henna plant contains biological properties that include antibacterial, anti-inflammatory, anti-cancer, antioxidant, and many more, according to numerous research. Henna leaves that have been dried can also be used as a significant cosmetic dye. The presence of phenolic, flavonoids, carbohydrates, alkaloids, tannins, protein, quinines, and coumarins was revealed by the pharmacological examination of henna extract. Henna's primary active ingredient is 2-hydroxy-1-4 naphthoquinone [6]. Trigonella foenum-graecum, often known as fenugreek, is a commonly planted annual herb in the Leguminosae family. in the Middle East, particularly in Egypt. Fenugreek is one of those plants whose leaves and seeds are frequently used as a spice in culinary dishes and as an ingredient in traditional medicine due to its potent flavor and perfume [7].

Thus, the purpose of this research is to investigate how applying Henna NPs can improve fenugreek growth as well as some of the plant's nutritional benefits.

MATERIALS AND METHODS

How to Make the Henna Extract Remedy put 100 ml of DI water and 10 g of henna powder in an Erlenmeyer flask. The mixture should be heated to 80 °C while being stirred 200 revolutions per minute for 20 minutes using a magnetic stirrer. The boiling henna solution was allowed to cool for thirty minutes before being filtered using Whitman No. 1 filter paper and cotton wool to create a fine henna solution. After that, it was kept at 4°C until it was required.

Synthesis of iron nanoparticles (FeNPs) [8]

Making nanoparticles using the green synthesis approach Fe ions were reduced and capped using henna

leaf extract. 20 ml of each 9 mM were mixed with 2 ml of extract to create sample solutions at a ratio of 1:10. Fe2SO4.7H2O solution in a 100mL Erlenmeyer flask water48 at room temperature, the solutions were constantly agitated at 60–70 °C for 8 hours, and then for an additional 24 hours at 37 °C .

FeNPs characterization

Synthesized FeNPs were characterized using the Making nanoparticles using the green synthesis approachFe ions were reduced and capped using henna leaf extract. 20 ml of each 9 mM were mixed with 2 mL of extract to create sample solutions at a ratio of 1:10. Fe2SO4•7H2O solution in a 100 mL Erlenmeyer flask water48 at room temperature. The solutions were constantly agitated at 60–70 °C for 8 hours, and then for an additional 24 hours at 37 °C .

Infrared spectroscopy (FTIR)

The sample is scanned using FTIR, which provides details on the pertinent functional groups and stability of the produced FeNPs. The resulting spectrum can be shown by a detector in the 4000–400 cm^{-1} range.

The FeNP samples were freeze-dried to a powder and then diluted 1:100 in potassium bromide for FTIR measurement. The acquired spectrum is within the wavelength range of 1000 to 3500 cm^{-1}56. The Bruker Germany Alpha FTIR spectrophotometer was used to perform FTIR spectroscopy [9].

XRD Analysis

Using a CuKa radiation source, Ni filter, and an X-ray diffractometer (30 kV/30 mA), the crystallinity of metallic nanoparticles was investigated. All powder forms nanoparticles in an X-ray diffraction sample were measured under experimental conditions at 2θ angle, with a range of 10 to 80° [10].

SEM Analysis

The SEM method was used to look at the morphology, size, and shape of the nanoparticles. The image was created using a finely ground powder of FeONPs and was captured using a JEOL JSM-6510LV scanning electron microscope at an accelerating voltage of 20 Kv [10].

Germination of Trigonella foenum-graecum L seeds

Pots' experiment was carried out in a Biology Department plastic house as a factorial experiment. Using the SEM method, the morphological characteristics, size, and form of the nanoparticles were investigated. Using a JEOL JSM-6510LV scanning electron microscope at a 20 kV accelerating voltage, a fine powder of FeO NPs was employed to create the image [11].

Germination of seeds of Trigonella foenum-graecumL

Pots experiment was carried out as a factorial experiment at the Biology Department's plastic house.

T1= control
T2=5mgl.l-1 FeSO4 NPs
T3=10mgl.l-1 FeSO4 NPs
T4= 75mg.l-1 GA3
T5=150mg.l-1 GA3
T6=T2 + T4
T7=T2 + T5
T8=T3 + T4
T9=T3 + T5

The parameters studied:

1. Plant height (cm)
2. Fresh weight (g)
3. Dry weight (g)
4. Weight of pod (g)
5. Weight of seeds (g)
6. Nitrogen concentration (%) [12].
7. Phosphorus concentration (%) [13].
8. Potassium concentration (%) [14].
9. Protein concentration (%) [15].

Three replicates were used in the design of the completely randomized design (CRD), and the findings were statistically assessed using the Least Significant Difference (LSD) test at a probability threshold of 5% [16].

Statistical evaluation

The experiments were conducted using the real experimental research design. SPSS version 22 was employed to analyze the information. $p< 0.05$ was considered significant.

RESULT AND DISCUSSION

Evaluation of FeO nanoparticle properties

One of the most useful methods for characterizing nanomaterials is the UV-Vis technique because nanomaterials have unique hues, which translate into unique peaks in this measurement. As a result, this test was performed, and the results demonstrated the existence of a peak at 291 nm, which is the iron oxide Figure 1 electron transition band [4].

Iron oxide nanoparticles were subjected to scanning electron microscopy (SEM) to ascertain their size, shape, and presence or absence in agglomerations. He measurement demonstrated the material's presence as tiny agglomerations with dimensions ranging from 31 to 53 nm, exhibiting irregular spherical geometric forms Figure 2.

The FTIR test revealed two broad bands at 3340 and 1643cm^{-1}, which are related to the presence of water. The first band is related to the hydroxyl group in water's stretching vibration, while the second band is related to the water's bending vibration. These peaks are present because the material will absorb moisture due to its large surface area. Additionally, as seen in Figure 3, the measurement revealed the distinctive iron oxide bands at 790, 1022, and 1516cm^{-1}, which are ascribed to the stretching vibration of Fe-O [3].

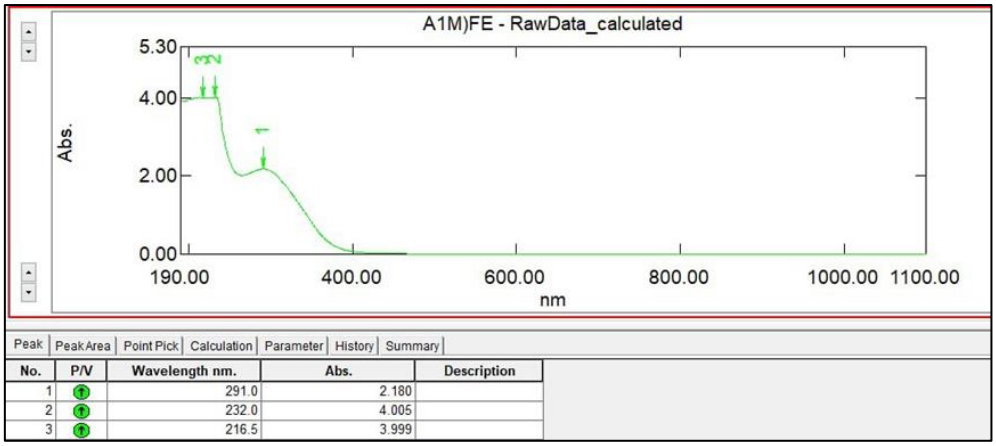

Figure 1: UV-Visible

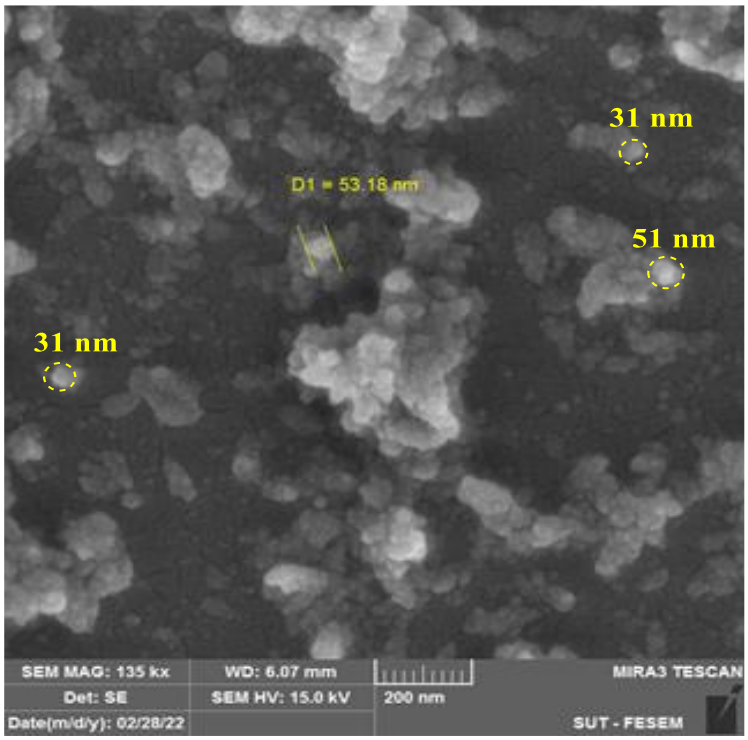

Figure 2: SEM

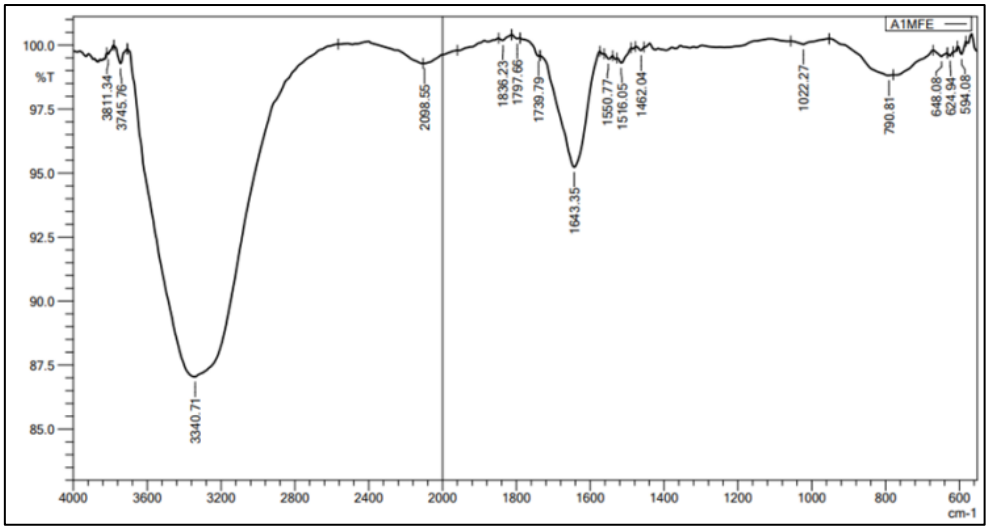

Figure 3: FTIR

XRD of FeO

The X-ray diffraction pattern of iron oxide nano powder revealed three primary peaks, located at 35.1110, 41.8003, and 61.9091 degrees. It is possible to assign these peaks to planes 3 1 1, 4 0 0, and 4 4 0, in that order. Furthermore, the Scherrer equation was used to determine the iron oxide nanoparticles' particle size. The results, which are displayed average of 21.26nm. Figure 4 and Table 1, indicated that the particle size ranged from 18.52 to 25.21.

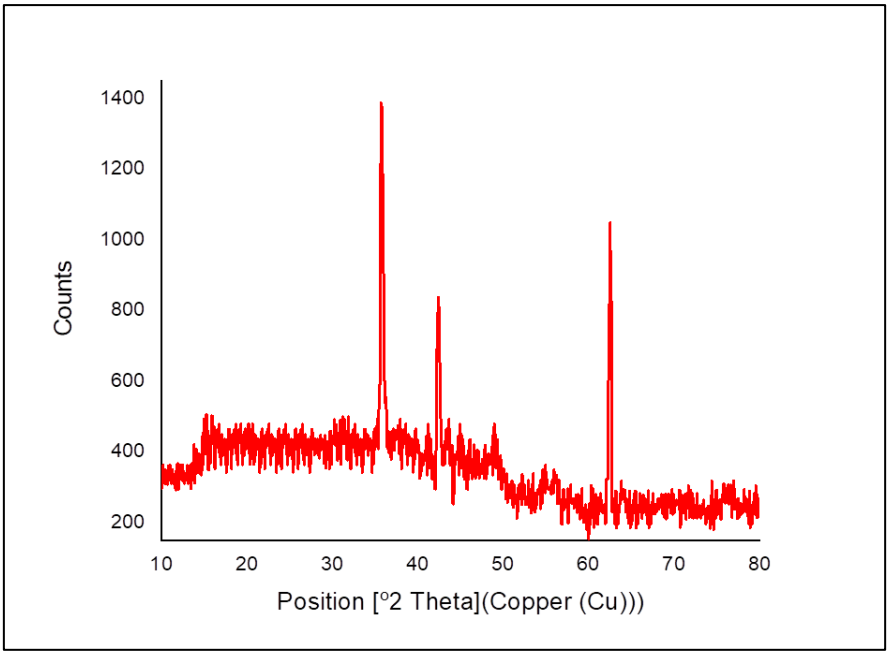

Figure 4: XRD of FeO nanoparticles

Table 1: XRD data of FeO nanoparticles

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]h k l	D (nm)	D average (nm)
35.1110	1240.1	0.43440	11	20.05
41.8003	691.2	0.48007400	18.52	
61.9091	896.7	0.38411440	25.21	

The statistical examination of the data in Tables 1 revealed that, when compared to the control (T1), which came in last, there was a significant difference between treatments at the 5% level. T8 outperformed the other treatments in terms of yield contents and all aspects of vegetative growth. The results of the T8 show that the plant grows to a height of 43.12 cm, weighs 30.41g fresh, 2.44g dry, 5.09g seed, and 7.39g pod at a rate of (55.56, 82.97, 162.36, 105.24, and 122.59)%, respectively. Additionally, the results showed that the interaction between the two research components (GA3 and FeSO4-NPs) improved urban growth and yield quality, yielding superior results than either factor alone. Table 1 Displayed the impact of FeSO4-NPs, GA3, and their combination on the vegetative development traits and yield contents of fenugreek contents. In comparison to the control (T1), which placed last, the T8 produced the following results: plant height of 43.12 cm, fresh weight of 30.41g, dry weight of 2.44g, seed weight of 5.09g, and pod weight of 7.39g at an increase rate of (55.56, 82.97, 162.36, 105.24, and 122.59)%, respectively, Additionally, the results showed that the interaction between the two research components (GA3 and FeSO4-NPs) improved urban growth and yield quality, yielding superior results than either factor alone.

Table 2 displays the effects of FeSO4-NPs, GA3, and their combination on the vegetative growth traits and yield contents of fenugreek. Table 2's results likewise demonstrated a significant difference at the 5% level between treatments, with treatment (T8) showing a much higher NPK and protein concentration than other treatments. In comparison to the control (T1), which came in last, the T8 produced N concentration of 4.1%, P concentration of 1.14%, K concentration of 4.22%, and protein concentration of 25.65% at an increase rate of (100.48, 395.65, 99.06, and 102.77)%, respectively.

Table 2's results further demonstrated that there was a significant difference (5% level) between treatments and that treatment (T8) outperformed other treatments in terms of NPK and protein concentration. N concentration was 4.1%, P concentration was 1.14%, K concentration was 4.22%, and protein concentration was 25.65% in the T8 group, with an increase rate of (100.48, 395.65, 99.06, and 102.77)%, respectively, in comparison to the control group (T1), which came in last. Furthermore, the findings showed that the combination of the two research components (FeSO4-NPs and GA3) produced higher values than either factor alone by

improving the concentration of protein and the urban nutrient state.

Table 2 effect concentrations of FeSO4 NPs, GA3 and interaction on some elements and protein concentration of fenugreek.Numerous factors, including increased CO2 fixation and elevated activity of the enzymes carbonic anhydrase and rubisco, may have contributed to the increased vegetative growth, nutritional status, and yield [18] increases membrane permeability for food uptake, triggers protein synthesis [19], speeds up the synthesis of IAA to encourage cell division and enlargement [20], and may lead to an increase in plant height and high dry mass accumulation. Also [21] states that the application of GA3 stimulates Florigen and breaks dormant flower buds to increase the number of blooms and subsequently the number of pods. Additionally, the movement of photosynthetic resources from the source to the sink (seeds) results in an increase in seed weight [22].

Nitrate reductase activity is enhanced by GA3 [23]. Moreover, could positively impact the accumulation of nutrients in the shoot and raise the content of protein [[24]. The majority of metabolic processes, including nitrogen fixation, chlorophyll biosynthesis, photosynthesis, respiration, and hormone biosynthesis, depend on iron (Fe). Moreover, it enhances non-enzymatic antioxidants and employs a cofactor for a large number of enzymes (about 140) [25].

Table 2: Treatment effect on the plant

Treatments	Plant Height (cm)	Fresh wt. (g)	Dry wt. (g)	Weight of seeds (g)	Weight of pod (g)
T1	27.72	16.62	0.93	2.48	3.32
T2	31.05	18.96	1.04	2.63	3.78
T3	33.25	20.77	1.31	3.02	4.29
T4	33.68	23.73	1.37	3.29	4.87
T5	36.89	26.03	1.59	3.71	5.47
T6	38.76	27.27	1.89	3.91	6.20
T7	39.80	28.48	2.03	4.31	6.89
T8	43.12	30.41	2.44	5.09	7.39
T9	40.73	28.47	2.09	4.27	6.42
LSD (0.05)	2.30	0.96	0.28	0.27	0.25

Enhancing vegetative growth and yield components may be caused by the beneficial effects of iron nanoparticles on plant growth and yield contents through increased synthesis of GA3 and jasmonic acid, the formation of chloroplasts, and the strengthening of the sink-source relationship [26]. Additionally, past research has shown that Fe-NPs are essential for directly and indirectly inducing antioxidant mechanisms, detoxifying reactive oxygen species, and maintaining osmotic equilibrium in plants contents [26]. In comparison to the control (T1), which placed last, the T8 produced the following results: plant height of 43.12 cm, fresh weight of 30.41g, dry weight of 2.44g, seed weight of 5.09g, and pod weight of 7.39g at an increase rate of (55.56, 82.97, 162.36, 105.24, and 122.59)%, respectively .

Additionally, the results showed that the interaction between the two research components (GA3 and FeSO4-NPs) improved urban growth and yield quality, yielding superior results than either factor alone.

Table 3 displays the effects of FeSO4-NPs, GA3, and their combination on the vegetative growth traits and yield contents of fenugreek. Table 3's results likewise demonstrated a significant difference at the 5% level between treatments, with treatment (T8) showing a much higher NPK and protein concentration than other treatments. In comparison to the control (T1), which came in last, the T8 produced N concentration of 4.1%, P concentration of 1.14%, K concentration of 4.22%, and protein concentration of 25.65% at an increase rate of (100.48, 395.65, 99.06, and 102.77)%, respectively. Additionally, the results showed that the combination of the two study components (GA3 and FeSO4-NPs) produced superior effects than either element alone by positively enhancing the urban nutritional condition and protein concentration.

Enhanced photosynthesis by stimulated Rubisco and carbonic anhydrase enzyme activity and increased CO2 fixation [20]; accelerates the synthesis of IAA to promote cell division and enlargement [21]; increases membrane permeability to uptake of nutrients and induces protein synthesis [17] are some of the possible causes of the increase in vegetative growth, nutrient state, and yield. Furthermore, GA3 application stimulates Florigen and breaks dormant flower buds to increase the number of flowers and subsequently the number of pods [11]. Additionally, photosynthetic materials are transferred from the source to the sink (seeds), increasing the weight of the seeds [22]. Nitrate reductase activity is enhanced by GA3 [9]. Moreover, could positively impact the accumulation of nutrients in the shoot and raise the content of protein [5].

The majority of metabolic processes, including nitrogen fixation, chlorophyll biosynthesis, photosynthesis, respiration, and hormone biosynthesis, depend on iron (Fe). Additionally, it enhances non-enzymatic antioxidants and employs a cofactor for a large number of enzymes (around 140 enzymes) [6].

Enhancing vegetative growth and yield components may be caused by the beneficial effects of iron nanoparticles on plant growth and yield contents through increased synthesis of GA3 and jasmonic acid, the formation of chloroplasts, and the strengthening of the sink-source relationship [26]. Additionally, past research has shown that Fe-NPs are essential for both direct and indirect induction of antioxidant systems, ROS detoxification, and osmotic balance protection as plants age [26].

Table 3: FeSO4 NPs, GA3, and their interactions' effect concentrations on various components and fenugreek protein concentration

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Protein (%)
T1	2.05	0.23	2.12	12.65
T2	2.27	0.40	2.15	14.11
T3	2.60	0.47	2.41	16.08
T4	2.81	0.60	2.80	17.57
T5	3.20	0.72	3.15	20.05
T6	3.44	0.85	3.24	21.39
T7	3.73	1.04	3.79	23.43
T8	4.11	1.14	4.22	25.65
T9	3.82	0.97	3.91	23.84
LSD (0.05)	0.20	0.14	0.22	0.25

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