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Effect of green synthesised iron nanoparticles on seedling traits in *Stylosanthes hamata*

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Stylosanthes hamata L. is a dichotomously branching, non-determinate, semi-erect, herbaceous annual to short-lived perennial pasture legume. The species is highly valued as a forage crop owing to its rich nutritional composition, containing 17–24% crude protein and 60–65% digestibility. Fiber content is relatively high, averaging 33% dry matter (DM) as crude fiber (24–49%), while lignin concentration is also substantial (14.60%) (Rao *et al*. 2004). Despite its desirable agronomic and nutritional attributes, the establishment of *S. hamata* through seeds remains challenging due to seed coat-imposed dormancy resulting from water-impermeable seed coats (hard-seededness), leading to erratic and non-uniform germination and stand establishment.

In recent years, nanopriming has emerged as an effective approach to enhance seed germination and early seedling growth. Nanopriming, a key application of nanotechnology in agriculture, has shown potential in improving seed vigour and breaking dormancy through improved water uptake, enzyme activation, and stress tolerance. Among the different synthesis approaches, green synthesis of nanoparticles offers several advantages over conventional chemical and physical methods. It is cost-effective, eco-friendly, operates under mild conditions, utilizes non-toxic materials, and is compatible with both agricultural and biomedical applications (Sandeep 2017). *Carica papaya* leaf extract, in particular, contains various bioactive compounds such as papain, alkaloids, flavonoids, saponins, and tannins, which possess antimicrobial, anti-inflammatory, and antioxidant properties (Mathew *et al*. 2017). These compounds act as natural reducing and stabilising agents during the synthesis of nanoparticles, facilitating a sustainable and biologically safe method for nanoparticle production.

Given the unique physiochemical properties of nanoparticles and their ability to interact with biological systems at the molecular level, nano-priming offers a promising technique to overcome seed dormancy and improve seedling vigour. The response, however, varies among species depending on their physiological characteristics and capacity to absorb and utilise nanoparticles. Therefore, it was hypothesised that seed treatment with green-synthesised iron nanoparticles could effectively break seed coat dormancy in *Stylosanthes hamata*, leading to improved germination and uniform seedling establishment. To test this hypothesis, the present investigation was undertaken to study the effect of green-synthesized iron nanoparticles on seed germination and seedling traits in *Stylosanthes hamata*.

The present study was carried out during 2023–24 at ICAR-Indian Grassland and Fodder Research Institute, Southern Regional Research Station (15°26'N, 75°07'E; at an elevation of 678 m amsl), Dharwad, while nanoparticle synthesis and characterization were performed at the Green Nanotechnology Laboratory, Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad, Karnataka.

Preparation of papaya leaf extract: Fresh, healthy leaves of *Carica papaya* were collected from the university campus and thoroughly washed with distilled water to remove surface impurities. 50 g of finely chopped leaves were added to 200 mL of distilled water in a beaker and boiled for 30 min. The mixture was then filtered through Whatman No. 1 filter paper, and the filtrate obtained was used as the reducing and stabilizing agent in the synthesis of iron nanoparticles.

Preparation of ferric chloride ($FeCl_3$) stock solution: Analytical grade ferric chloride ($FeCl_3$) was used as the precursor for nanoparticle synthesis. A 100 ppm stock solution was prepared by dissolving 0.1 g of $FeCl_3$ in one litre of distilled water.

Greens synthesis of iron nanoparticles: For the synthesis of green iron nanoparticles (FeNPs), 25 mL of freshly

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prepared 100 ppm $FeCl_3$ solution was added dropwise to 25 mL of papaya leaf extract in a 1:1 ratio under continuous stirring using a magnetic stirrer. The reaction mixture was maintained at 80°C for 4 h. A visible colour change from light green to blackish indicated the formation of iron nanoparticles. The synthesised FeNPs were characterized using Ultraviolet-Visible (UV-Vis) Spectrophotometer, Particle Size Analyzer (PSA), Atomic Force Microscope (AFM), and Scanning Electron Microscope (SEM) to confirm their formation, morphology, and size distribution.

Seed material and treatments: Freshly harvested seeds of *Stylosanthes hamata* were obtained from ICAR-Indian Grassland and Fodder Research Institute, Dharwad, Karnataka. The seeds were subjected to nano-priming by soaking them in green-synthesised iron nanoparticle suspensions of varying concentrations for 12 h. The treatments included: T_1, Control (untreated); T_2, Mechanical scarification with sandpaper (5 min); T_3, Seed priming with FeNPs at 20 ppm; T_4, Seed priming with FeNPs at 40 ppm; T_5, Seed priming with FeNPs at 60 ppm; T_6, Seed priming with FeNPs at 80 ppm; T_7, Seed priming with FeNPs at 100 ppm; T_8, Seed priming with FeNPs at 120 ppm; and T_9, Hydro-priming (distilled water). Each treatment was replicated thrice. The experiment was laid out in a completely randomised design (CRD). Data were statistically analysed using the F-test at the 1% level of significance ($p \leq 0.01$) as per the procedures described by Gomez and Gomez (1984). Treatment means were compared using the Critical Difference (CD) test. The seed quality parameters were assessed following the International Seed Testing Association (ISTA 2013) rules using the between-paper (BP) method. Germination counts were taken on the 14th day after incubation. The observations record included: Germination percentage (%), abnormal seedlings (%), dead seeds (%), hard seeds (%), shoot length (cm), root length (cm), speed of germination, seedling vigour index (SVI), seedling dry weight (mg) and electrical conductivity (dS/m).

Papaya leaf extract served as both a reducing and capping agent during the synthesis process. The colour change of the reaction mixture from light green to greyish-black confirmed the reduction of iron ions (Fe^{3+}) to Fe^0 nanoparticles, attributed to the excitation of surface plasmon vibrations (Yehia and Ali 2020, Lakshmnarayanan *et al*. 2021). The phytochemicals and bioactive compounds in papaya leaf extract especially phenolic compounds played a key role in this process, as their hydroxyl groups exhibited strong antioxidative and reducing properties (Mathew *et al*. 2017).

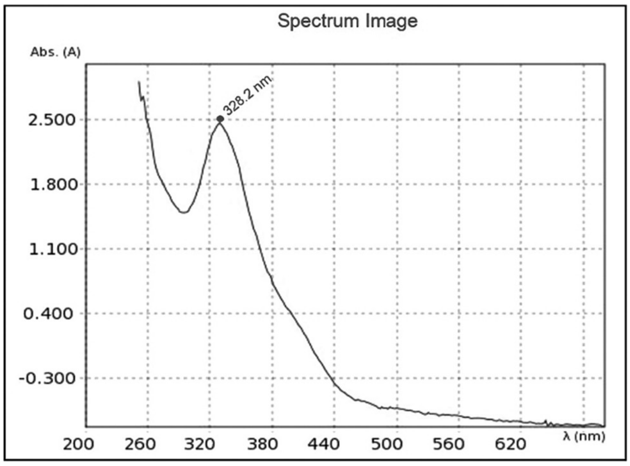


Fig. 1a Papaya leaves based FeNPs UV-328.2 nm.

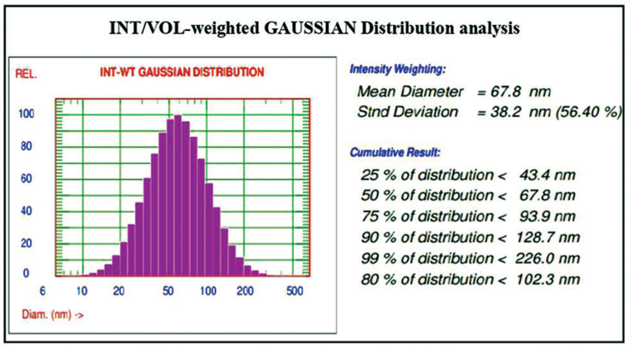


Fig. 1b Papaya leaves based FeNPs PSA-67.8 nm.

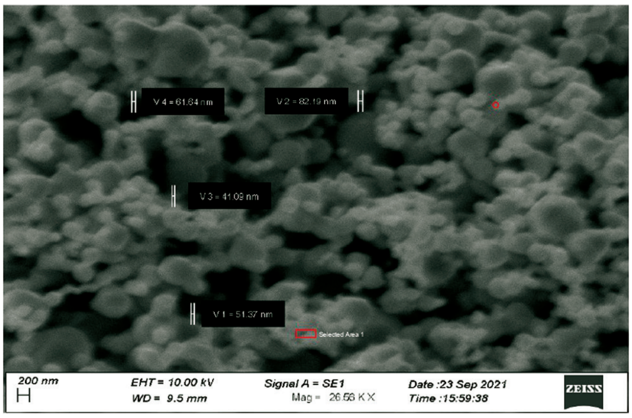


Fig. 1c SEM image of papaya leaf based FeNPs.

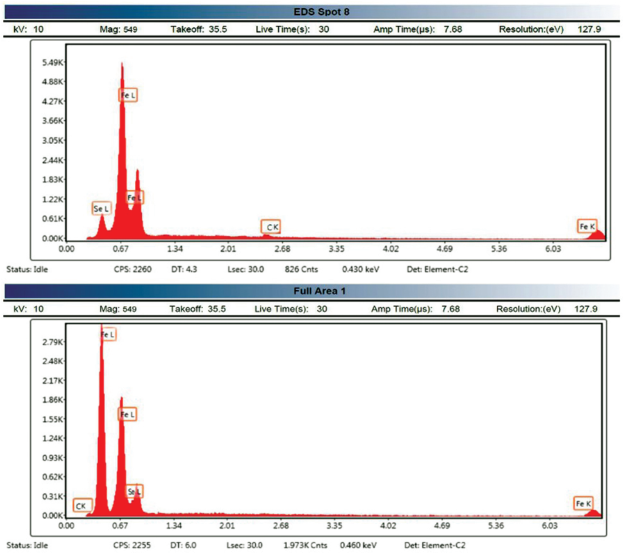


Fig. 1d EMAX analyzed graphs of papaya leaf based FeNPs.

Fig. 1 Characterisation of green-synthesised iron nanoparticles.

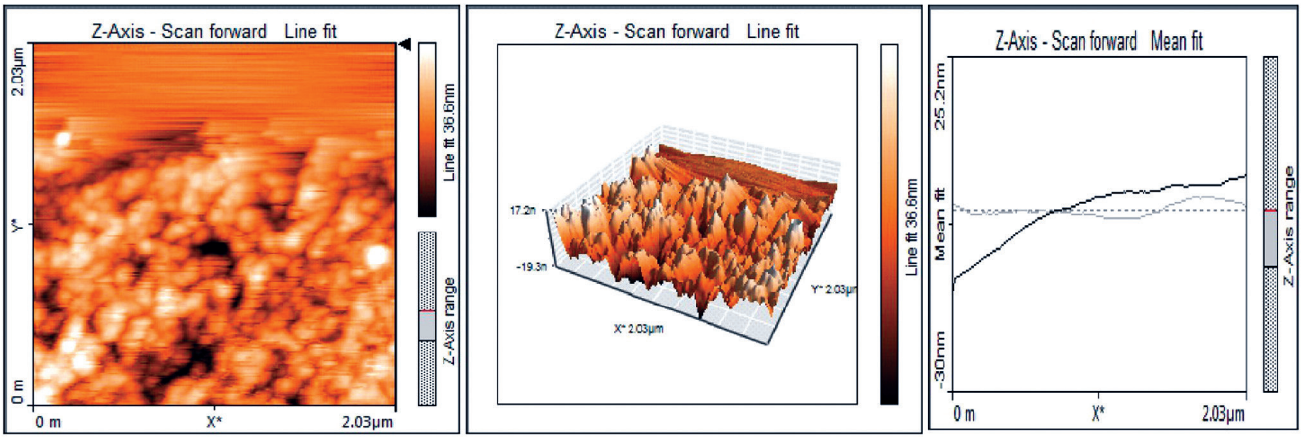


Fig. 2 AFM images of papaya leaf based FeNPs.
Two, three and graphical images of synthesised iron nanoparticles from papaya leaf extracts- 67.8 nm. AFM, Atomic force microscopy.

Characterisation of green-synthesised iron nanoparticles

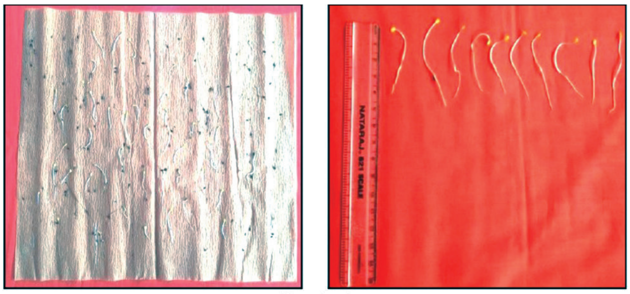
UV-Visible spectroscopy: The formation of FeNPs was preliminarily confirmed by UV-Visible spectroscopy in the wavelength range of 200–700 nm (Fig. 1a). A distinct absorption peak was observed at 328.2 nm, corresponding to the surface plasmon resonance of iron nanoparticles. Similar absorption behaviour was reported by Saranya *et al*. (2017) and Lakshmnarayanan *et al*. (2021), confirming successful nanoparticle synthesis.

Particle size analysis: The particle size distribution and intensity-weighted mean diameter of FeNPs were determined using a Particle Size Analyzer (Fig. 1b). The average particle size was found to be 67.8 nm, corroborating the findings of Sandeep (2017), who also reported nanoscale FeNPs synthesised via green methods.

Scanning electron microscopy (SEM): Surface morphology of the synthesised FeNPs, analysed by SEM (Fig. 1c) revealed predominantly spherical-shaped particles. These observations are consistent with those of Lakshmnarayanan *et al.* (2021), who synthesised iron nanoparticles using *Bauhinia tomentosa* leaf extract with an average particle size of approximately 70 nm. Elemental composition, confirmed through Energy Dispersive X-ray Spectroscopy (EDS), verified the presence of iron as the principal element (Fig. 1d) (Aisida *et al*. 2021).

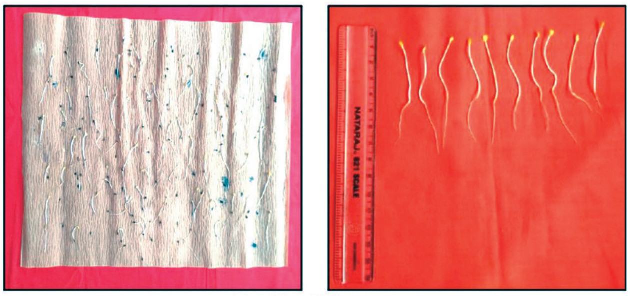
Atomic force microscopy (AFM): AFM images (Fig. 2) revealed the surface topology of the synthesised FeNPs, displaying smooth surfaces with negligible flocculation. These findings were in line with those of Aisida *et al.* (2021), who reported similar surface characteristics for FeNPs synthesised from mango leaf extract.

Effect of iron nanoparticles on seedling traits: Seed quality parameters of *Stylosanthes hamata* showed significant variation due to seed priming with green-synthesised iron nanoparticles (Table 1). Among the treatments, FeNPs at 80 ppm (T_6) recorded the highest germination percentage (59.67%), followed by 100 ppm (T_7, 56.67%), whereas the control (T_1) exhibited the lowest germination (40.33%) (Fig. 3). A minimum proportion of abnormal seedlings was recorded in T_3 (1.67%), followed by T_4 (2.67%), while mechanical scarification (T_2) resulted in the highest proportion (19.00%). Interestingly, T_2 also showed the lowest percentage of hard seeds (15.67%) due to partial removal of the impermeable seed coat, while the



Control

FeNPs 80 ppm



FeNPs 120 ppm

Fig. 3 Effect of nano priming on germination and seedling length of *Stylosanthes hamata.*

Table 1 Effect of green synthesised iron nanoparticles for seedling traits in *Stylosanthes hamata*

Treatments	Germination (%)	Abnormal seedlings (%)	Hard seeds (%)	Dead seeds (%)	Shoot length (cm)	Root length (cm)	Seedling vigour index-I	Speed of Germination	Seedling vigour index-II	Seedling dry weight (mg)	Electric conductivity (dS/m)
T_1	40.33 (39.41)*	7.00 (15.34)*	47.67 (43.65)*	5.00 (12.92)*	2.09	2.04	166	8.40	917	22.73	0.074
T_2	53.33 (46.89)	19.00 (25.83)	15.67 (23.29)	12.00 (20.26)	2.51	2.53	269	11.22	1293	24.27	0.160
T_3	49.67 (44.79)	1.67 (7.33)	45.67 (42.50)	3.00 (9.97)	3.54	3.15	332	13.24	1441	29.03	0.079
T_4	51.33 (45.75)	2.67 (9.36)	42.33 (40.57)	3.67 (11.01)	3.68	3.48	368	14.08	1580	30.80	0.091
T_5	55.67 (48.24)	3.00 (9.97)	36.33 (37.05)	5.00 (12.92)	3.81	3.67	416	14.64	1739	31.25	0.101
T_6	59.67 (50.56)	4.00 (11.53)	29.33 (32.77)	7.00 (15.34)	4.32	4.13	504	15.59	2042	34.23	0.113
T_7	56.67 (48.81)	7.00 (15.35)	28.33 (32.14)	8.00 (16.42)	4.07	3.71	441	14.87	1893	33.40	0.136
T_8	48.33 (44.03)	13.00 (21.13)	27.67 (31.72)	11.00 (19.36)	3.29	3.14	311	11.33	1308	27.07	0.143
T_9	42.00 (40.38)	7.33 (15.70)	45.33 (42.31)	5.33 (13.34)	3.04	3.12	259	9.35	1061	25.27	0.077
SEm ±	0.49	0.36	0.52	0.22	0.064	0.065	8.73	0.23	27.00	0.50	0.002
CD at 1 %	1.48	1.07	1.54	0.67	0.191	0.196	26.15	0.70	80.83	1.48	0.005

*Figures in parenthesis indicates act sine transformed values. Refer to methodology for treatment details.

control (T_1) recorded the highest (47.67%). The lowest proportion of dead seeds was recorded in T_3 (3.0%) and T_4 (3.67%), whereas T_2 had the highest (12.0%).

The highest speed of germination (15.59) was observed in T_6, followed by T_7 (14.87), compared to a much lower value in the control (8.40). Similarly, T_6 recorded the highest shoot length (4.32 cm) and root length (4.13 cm), followed by T_7 (4.07 cm and 3.71 cm, respectively). Correspondingly, seedling vigour indices I and II were maximum in T_6 (504 and 2042, respectively), followed by T_7 (441 and 1893), while the control recorded the lowest values (166 and 917). Seedling dry weight was also highest in T_6 (34.23 mg), followed by T_7 (33.40 mg), compared to the lowest in the control (22.73 mg). Electrical conductivity (EC) was lowest in the control (0.074 dS/m), indicating better membrane stability, and highest in mechanically scarified seeds (T_2, 0.160 dS/m), suggesting increased membrane leakage due to physical injury (Table 1).

Seeds primed with FeNPs at 80 ppm (T_6) exhibited superior performance in terms of germination, seedling growth, and vigour indices. Iron plays a critical role as a cofactor for several enzymes involved in respiration and energy metabolism during germination. The FeNPs likely adhered to the seed coat and facilitated enhanced penetration, improving water uptake and enzymatic activation. Similar results were reported by Remya (2016) in beans and Tovar *et al*. (2020) in maize, where FeNP treatment enhanced germination speed through nanopore formation and up-regulation of aquaporin genes, resulting in accelerated water absorption and metabolic activity.

The increase in shoot and root length (Fig. 3), as well as seedling dry weight in T_6, might be attributed to improved cellular elongation and metabolic stimulation. Kim *et al*. (2014) observed similar enhancement in *Arabidopsis thaliana*, and Sutariya *et al*. (2021) reported comparable results in rice, linking FeNP treatment to cell wall loosening and elongation. Lower percentages of abnormal and dead seedlings in FeNPs 40 ppm (T_4) treatment suggest reduced oxidative stress and improved membrane integrity, whereas mechanical scarification (T_2) caused higher abnormality and mortality due to physical injury to the embryo and seed coat. This agrees with the findings of Martin and Cuadra (2004). The high proportion of hard seeds in the control (T_1) confirmed the physical dormancy characteristic of *Stylosanthes* spp., whereas mechanical scarification (T_2) significantly reduced hard seeds through abrasion of the seed coat, results consistent with Bhatt *et al*. (2008).

Higher EC values in T_2 and T_8 (FeNPs 120 ppm) indicated increased membrane permeability and electrolyte leakage, suggesting structural damage or oxidative stress at higher nanoparticle concentrations (Jin *et al*. 2006). While moderate concentrations (80–100 ppm) of FeNPs enhanced germination and seedling traits, further increase in concentration ($\geq$120 ppm) resulted in reduced performance, possibly due to the generation of reactive oxygen species (ROS) leading to oxidative stress, as reported by Guha *et al*. (2018) in rice cultivars.

SUMMARY

The experiment was conducted to evaluate the effect of green-synthesised iron nanoparticles (FeNPs) using papaya (*Carica papaya*) leaf extract on seed dormancy and seedling traits of *Stylosanthes hamata*, an important forage legume. The FeNPs exhibited an absorption peak at 328.2 nm in the UV-Vis spectrum and an average particle size of 67.8 nm as determined by PSA. Seed priming with green-synthesised FeNPs significantly influenced the seed quality parameters of *S. hamata*. Among the treatments, FeNPs at 80 ppm (T_6) recorded the highest seed germination (59.67%), root length (4.13 cm), shoot length (4.32 cm), seedling vigour index-I (504), speed of germination (15.59), and seedling dry weight (34.23 mg), along with a lower proportion of hard seeds (29.33%) and abnormal seedlings (4%), compared to the control. However, higher concentrations of FeNPs (100 ppm and 120 ppm) adversely affected germination and seedling growth. Therefore, green-synthesised iron nanoparticles can be effectively used as an alternative, eco-friendly seed treatment method to overcome hard seed coat dormancy in *Stylosanthes hamata*.

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