Foliar Application of Nano-Iron Oxide for Improving Chlorophyll Content and Yield in *Arachis hypogaea* L.

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ABSTRACT

Background: Iron is one of the most important components of chlorophyll biosynthesis, electron transport mechanism, and enzyme activity in plants, however, the availability of iron in alkaline soils is limited, making iron deficiency a serious concern in groundnut cultivation. Conventional iron sources do not solve the issue of chlorosis due to their low solubility, high oxidation rate, and low penetration on the leaf surface.

Objective: The objective of the current study is to analyze the potential of nano-Fe_2O_3 for chlorophyll production and photosynthetic processes.

Methods: A field trial was organized in randomized complete block fashion with 7 treatments – control, recommended dosage of $FeSO_4$, 4 concentrations of nano-Fe_2O_3 (25, 50, 75, and 100 ppm), and combined nano-Fe_2O_3 dosage replicated thrice. Chlorophyll content, SPAD readings, gas-exchange parameters, growth parameters, leaf/seed iron content, and yield parameters were analyzed using ANOVA and Tukey's HSD test ($P < 0.05$).

Results: Nano-Fe_2O_3 at the concentration of 75 ppm had the maximum values of total chlorophyll content, SPAD value, net photosynthetic rate, iron concentration in the leaves and seeds, and seed yield, which was considerably larger than the values for the control and classical $FeSO_4$ treatments, while the combined method proved to have comparable or at least marginally higher performance.

Conclusion: Using nano-iron oxide as a foliar treatment means utilizing iron more efficiently and achieving greater photosynthetic activity than with conventional fertilizers, which will ultimately involve getting higher yields and making this treatment a valuable addition in the sustainable management of trace micronutrients for groundnut growing.

Keywords: Groundnut, Nano-Iron Oxide (Fe_2O_3), Foliar Fertilization, Iron Use Efficiency, Photosynthesis, Chlorophyll

1. Introduction

Arachis hypogaea L. (also known as groundnut or peanut) is one of the most crucial edible legumes and oilseeds in the world. It is cultivated in more than 100 countries, especially in the semi-arid tropical regions, and is essential for edible oil, protein and biodiesel industries [1]. In addition to its rich nutrient profiles such as oil, micronutrients and protein, groundnut also helps stabilize the farmers' incomes and improves soil nutrients via biological nitrogen fixation [2].

Iron (Fe) is an essential micronutrient for metabolism, as it acts as a cofactor of many enzymes involved in chlorophyll formation, cytochromes, ferredoxins and overall photosynthesis process [3]. Iron deficiency usually results in interveinal chlorosis, low chlorophyll levels, decreased efficiency of photosynthesis and can cause yield losses; this deficiency is particularly common in calcareous, alkaline and sandy soils where Fe^{3+} becomes precipitated in the form of oxide [4]. The use of this crop in such soils makes it very susceptible to iron deficiency chlorosis.

Traditional correction is based on applying inorganic salts (like $FeSO_4$) and chelates (such as Fe-EDTA) either through the soil or the leaves. The effectiveness of these methods is constrained by rapid oxidation into forms that are unavailable, poor cuticular penetration, limited mobility within the plant, and the requirement for frequent and expensive applications. Unlike conventional fertilizers, nanotechnology provides an alternative for effective micronutrient management. Nanoparticles (Fe_2O_3/Fe_3O_4, typically within the size range of 20-50 nm) have a high surface area to volume ratio and a high reactivity as well as the ability to penetrate through the cuticle and stomata effectively, thus allowing more effective absorption and movement with little application.

In spite of the interest in nano-fertilizers, there are very few empirical studies on their effectiveness on the groundnut crop. Therefore, this study aims at studying the effect of foliar application of nano-Fe_2O_3 at different concentrations and with conventional fertilizer on chlorophyll production, photosynthesis, growth, iron supply to the plant and pod yield.

2. Literature Review

Iron nutrition in legumes has been widely studied given the crop group's high Fe demand for nodulation and nitrogenase activity, with deficiency shown to depress both symbiotic nitrogen fixation and chlorophyll formation [2, 3]. Foliar fertilization has been

established as an efficient route for correcting micronutrient deficiencies because it bypasses soil fixation reactions that limit root uptake, though efficacy depends strongly on formulation chemistry [5].

Several investigations report that nano-formulated micronutrients outperform bulk salts in uptake efficiency. Nano-Fe_2O_3 and Fe_3O_4 have been associated with improved chlorophyll a and b content, elevated SPAD readings, and enhanced photosynthetic rate in cereals and legumes, attributed to greater particle penetration through stomata and the cuticle [6, 8]. Mechanistically, nanoparticles are thought to donate Fe more readily to protochlorophyllide reductase and other Fe-dependent enzymes central to tetrapyrrole biosynthesis [3, 9].

Reviews of nanotechnology in agriculture highlight improved micronutrient use efficiency, reduced total nutrient input, and lower runoff losses as key sustainability advantages of nano-fertilizers relative to conventional broadcast or foliar salts [7, 10]. Yield enhancement following nano-fertilizer application has been documented across multiple crops, generally linked to improved source–sink relationships driven by enhanced photosynthetic capacity [11, 12].

Nonetheless, concerns persist regarding nanoparticle dose-dependent phytotoxicity, translocation behavior, and long-term soil and ecological effects, indicating that concentration optimization is essential [13]. Research gaps specific to groundnut remain: prior work has concentrated on cereals (rice, wheat, maize), leaving the dose–response relationship, optimal spray schedule, and combined nano–conventional fertilizer strategies in *Arachis hypogaea* comparatively unexplored [14].

3. Materials and Methods

3.1. Experimental Site

The field trial was conducted during the *kharif* (rainy) season at a research farm located in a semi-arid agro-climatic zone (approximate coordinates 22.5°N, 75.9°E), characterized by sandy loam soil (pH 7.8, organic carbon 0.42%, DTPA-extractable Fe 3.1 mg kg^{-1}) and a mean seasonal rainfall of 650 mm with temperatures ranging from 24–36°C.

3.2. Experimental Design

A randomized complete block design (RCBD) with seven treatments and three replications (21 plots total) was used. Each plot measured 4 m × 3 m, and the crop duration spanned 110–115 days from sowing to harvest.

3.3. Nano-Iron Oxide Characteristics

Nano-Fe_2O_3 (>99% purity, hexagonal/rhombohedral crystal structure, spherical morphology, particle size 20–50 nm confirmed by TEM and XRD) was procured from a certified nanomaterial supplier and used as a stable aqueous colloidal suspension.

3.4. Fertilizer Treatments

Treatments were: T1 – control (water spray); T2 – recommended $FeSO_4$ (0.5% foliar spray); T3–T6 – nano-Fe_2O_3 at 25, 50, 75, and 100 ppm; T7 – nano-Fe_2O_3 (75 ppm) + recommended $FeSO_4$ dose. Three foliar sprays were applied at 30, 45, and 60 days after sowing (DAS), corresponding to vegetative, flowering, and pegging stages, at a spray volume of 500 L ha^{-1} using a knapsack sprayer during early morning hours (Table 1).

Table 1: Experimental treatments, foliar nano-Fe_2O_3 concentrations, and application schedule

Treatment	Description	Concentration	Spray stages (DAS)
T1	Control	Water spray	30, 45, 60
T2	Conventional Fe	$FeSO_4$ 0.5%	30, 45, 60
T3	Nano-Fe_2O_3	25 ppm	30, 45, 60
T4	Nano-Fe_2O_3	50 ppm	30, 45, 60
T5	Nano-Fe_2O_3	75 ppm	30, 45, 60
T6	Nano-Fe_2O_3	100 ppm	30, 45, 60
T7	Nano-Fe_2O_3 + $FeSO_4$	75 ppm + 0.5%	30, 45, 60

3.5. Crop Information

The groundnut variety used was a widely cultivated Spanish-bunch type (TAG-24 equivalent), sown at 30 cm × 10 cm spacing with certified seed. Irrigation followed a life-saving schedule at critical growth stages (flowering, pegging, pod development); standard recommended practices governed weed and pest management.

3.6. Growth, Physiological, Yield, and Nutrient Measurements

Plant height, branch number, leaf area index, and dry matter accumulation was recorded at 60 and 90 DAS. Chlorophyll a, b, total chlorophyll, and carotenoids were estimated spectrophotometrically (DMSO extraction); SPAD-502 readings, net photosynthetic rate, stomatal conductance (portable infrared gas analyzer), and relative water content were measured at 75 DAS. At harvest, pods per plant, pod weight, shelling percentage, hundred-seed weight, seed yield, biological yield, and harvest index were recorded. Leaf and seed Fe concentration were determined by Atomic Absorption Spectroscopy (AAS) following di-acid digestion, and iron uptake and use efficiency were calculated.

3.7. Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA) appropriate to RCBD, and treatment means were separated using Tukey's Honestly Significant Difference (HSD) test at $P < 0.05$, using R software (v4.3).

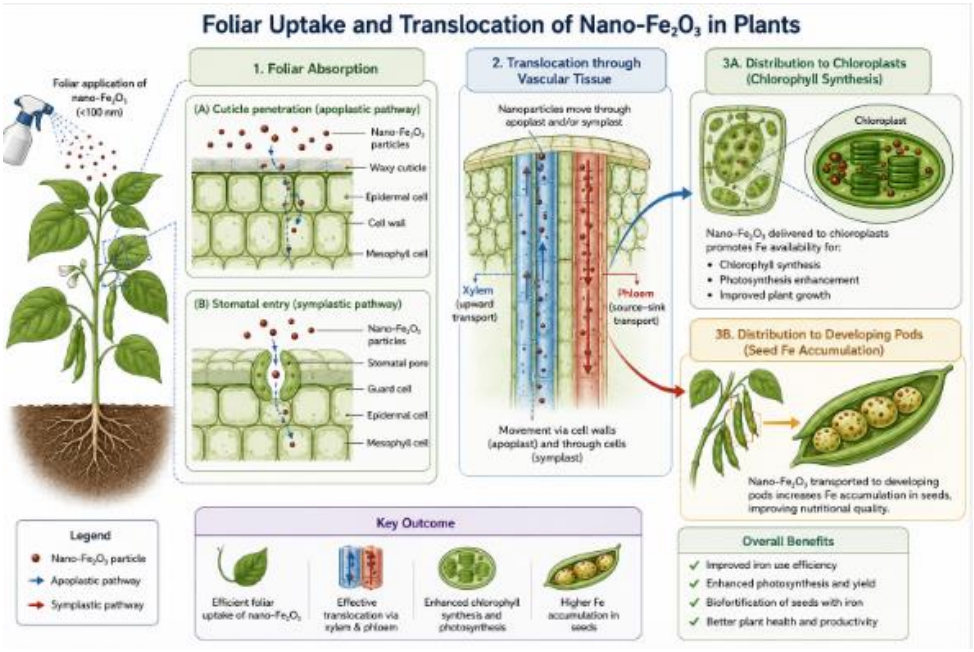

Fig 1: Schematic illustrating foliar absorption of nano-Fe_2O_3 through the leaf cuticle and stomata, apoplastic/symplastic translocation via vascular tissue, and subsequent distribution to chloroplasts (for chlorophyll synthesis) and developing pods (for seed Fe accumulation).

4. Results

4.1. Growth Performance

Plant height, LAI, and dry matter accumulation increased progressively with nano-Fe_2O_3 concentration up to 75 ppm before plateauing at 100 ppm. T5 (75 ppm) recorded the tallest plants (48.6 cm) and highest LAI (3.42) at 90 DAS, significantly higher than the control (38.2 cm; LAI 2.51) and T2 (42.9 cm; LAI 2.88) ($P < 0.05$). Branch number followed a similar trend, with T7 marginally exceeding T5.

4.2. Chlorophyll and Physiological Responses

Total chlorophyll content rose from 1.42 mg g^{-1} FW (control) to 2.36 mg g^{-1} FW in T5, a 66% increase, with SPAD values increasing correspondingly from 38.4 to 52.7 (Table 2). Chlorophyll a and b both increased significantly in nano-treated plots relative to T2, indicating superior pigment biosynthesis. Net photosynthetic rate improved from 14.8 to 23.1 µmol CO_2 m^{-2} s^{-1}, alongside higher stomatal conductance and relative water content, in T5 relative to the control.

4.3. Yield Performance

Seed yield increased from 1.68 t ha^{-1} (control) to 2.74 t ha^{-1} in T5 (63% increase) and 2.81 t ha^{-1} in T7, both significantly higher than T2 (2.11 t ha^{-1}). Pod number, pod weight, hundred-seed weight, and harvest index followed parallel improvements, while shelling percentage showed smaller, non-significant differences among treatments beyond T3.

4.4. Iron Nutrition

Leaf Fe concentration increased from 68 mg kg^{-1} (control) to 142 mg kg^{-1} in T5, and seed Fe concentration rose from 34 to 58 mg kg^{-1}. Iron use efficiency was highest in T5, exceeding T2 by approximately 35%, indicating more efficient nanoparticle-mediated Fe assimilation per unit applied.

Table 2: Effects of foliar nano-Fe_2O_3 application on chlorophyll, physiological, iron nutrition, and yield attributes of *Arachis hypogaea* L.

Parameter	T1 (Control)	T2 ($FeSO_4$)	T5 (Nano 75 ppm)	T7 (Nano+$FeSO_4$)
Total chlorophyll (mg g^{-1} FW)	1.42	1.79	2.36	2.41
SPAD value	38.4	44.6	52.7	53.5
Net photosynthesis (µmol CO_2 m^{-2} s^{-1})	14.8	18.2	23.1	23.6
Leaf Fe (mg kg^{-1})	68	96	142	148
Seed Fe (mg kg^{-1})	34	43	58	60
Seed yield (t ha^{-1})	1.68	2.11	2.74	2.81
Harvest index (%)	31.2	34.5	39.8	40.3

Values represent treatment means (n = 3); LSD/Tukey grouping (not shown) indicated T5 and T7 formed a statistically homogeneous top group, significantly superior to T1–T3 ($P < 0.05$).

5. Discussion

The superior performance of nano-Fe_2O_3 relative to conventional $FeSO_4$ is consistent with its proposed mechanism of enhanced cuticular and stomatal penetration, which allows more Fe to reach mesophyll cells before oxidative precipitation can occur, a limitation intrinsic to bulk ionic Fe sprays [4, 6]. The dose-dependent chlorophyll increases up to 75 ppm, followed by a plateau at 100 ppm, suggests that Fe supply beyond the enzymatic demand of the chlorophyll biosynthetic pathway (particularly protochlorophyllide oxidoreductase) yields diminishing marginal returns, echoing the saturation kinetics reported for other Fe-dependent metabolic enzymes [3, 9].

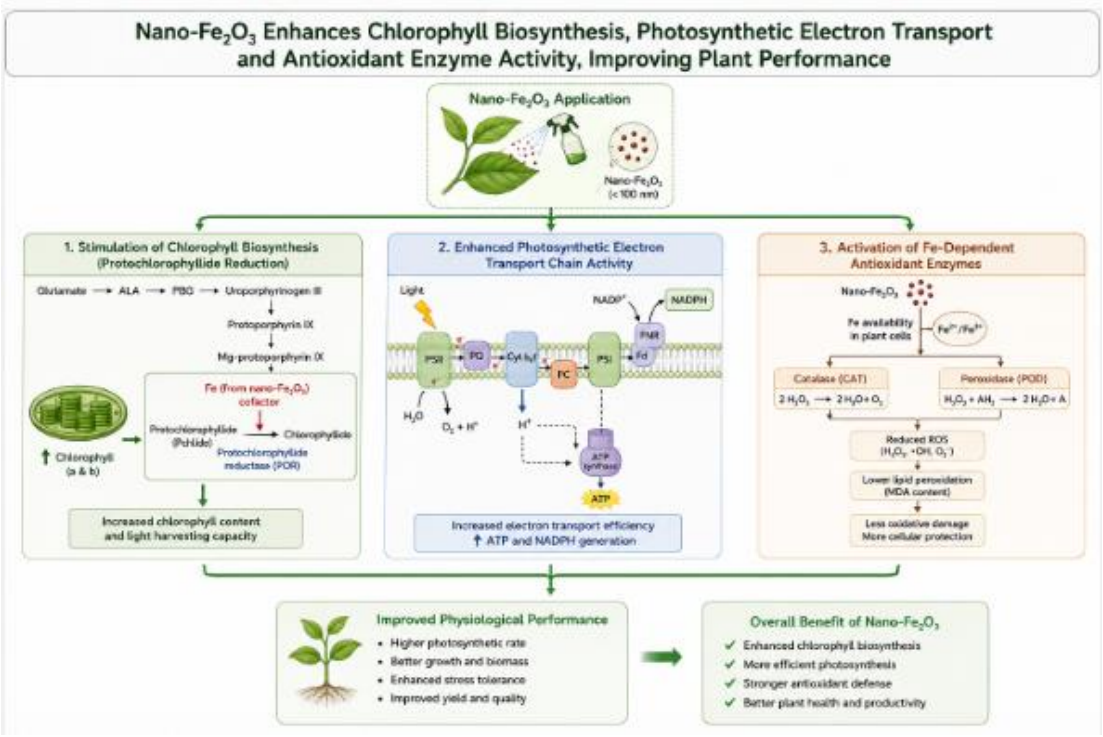

Fig 2: Conceptual diagram depicting nano-Fe_2O_3-mediated stimulation of chlorophyll biosynthesis (protochlorophyllide reduction), enhanced photosynthetic electron transport chain activity, and activation of Fe-dependent enzymes (catalase, peroxidase), collectively improving physiological performance.

The tight coupling observed between chlorophyll enhancement, photosynthetic rate, and seed yield supports the view that improved source capacity (chlorophyll-driven carbon assimilation) directly underpins sink strength (pod filling), a relationship previously reported for nano-enabled micronutrient interventions in other crops [11, 12]. The marginal but consistent advantage of the combined nano + conventional treatment (T7) over nano alone (T5) indicates a potential additive, rather than purely substitutive, role for nanoparticle formulations within existing fertilization programs.

From a sustainability standpoint, the substantially lower nano-Fe_2O_3 concentrations required to match or exceed conventional fertilizer performance imply reduced total micronutrient input and associated environmental loading, aligning with broader claims about nano-fertilizer efficiency [7, 10]. However, excessive nanoparticle application carries documented risks of phytotoxicity and unknown long-term soil ecological effects, reinforcing the necessity of concentration optimization rather than indiscriminate scaling [13]. Economic feasibility will depend on nanomaterial production costs relative to yield gains; as synthesis methods mature, cost parity with conventional fertilizers appears increasingly plausible [14].

This study's limitations include a single-season, single-location trial and the absence of soil residual nanoparticle tracking. Future work should examine multi-season validation, nanoparticle fate in soil and non-target organisms, and integration with precision-agriculture delivery systems for site-specific micronutrient management.

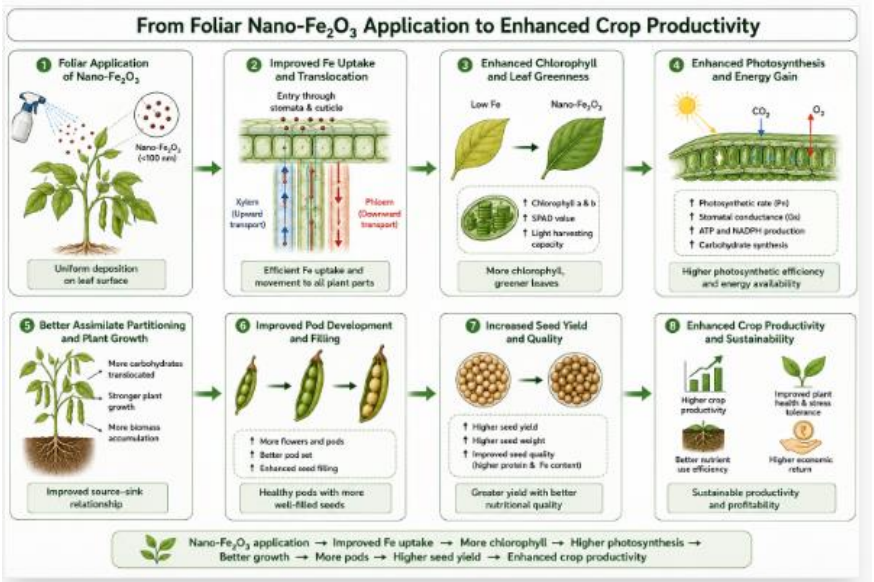

Fig 3: Graphical workflow summarizing the cascade from foliar nano-Fe_2O_3 application through improved Fe uptake, chlorophyll enhancement, and photosynthetic gain to pod development, seed yield, and overall crop productivity.

6. Conclusion

The use of nano-Fe_2O_3 application on groundnut trees, especially at 75 ppm improved chlorophyll content, photosynthetic ability, iron absorption, pod yield as compared to both control (without treatment) and conventional treatment (using $FeSO_4$), whereby the combined treatment of nano and conventional fertilization offered little additional benefits. Consequently, nano-iron oxide offers great potential to produce higher physiological and yield results at relatively lower application doses, thus proving its feasibility in sustainable resource-efficient micronutrient management in groundnut cultivation. However, more locations need to be tested in order to confirm the feasibility and effectiveness of this innovative technology.

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