Evaluation of Nano-Phosphorus Fertilizers for Improving Phosphorus Use Efficiency in Chickpea

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ABSTRACT
Background: Chickpea (*Cicer arietinum* L.) is the second most widely cultivated grain legume worldwide, and its productivity is strongly constrained by phosphorus (P) deficiency in semi-arid and calcareous soils. Conventional phosphatic fertilizers exhibit low phosphorus use efficiency (PUE) because a large proportion of applied phosphorus is rapidly fixed into insoluble calcium, iron, or aluminium phosphates, rendering it unavailable to roots and contributing to eutrophication of downstream water bodies.
Objective: This study was undertaken to synthesise and characterise a nano-phosphorus (Nano-P) fertilizer and to evaluate its effect on growth, physiology, nutrient uptake, yield attributes, phosphorus use efficiency, and soil health of chickpea relative to the recommended dose of conventional phosphorus (RDP).
Methods: A hydroxyapatite-based Nano-P fertilizer was synthesised by wet-chemical co-precipitation and characterised using SEM, TEM, XRD, FTIR, DLS, and zeta potential analysis. A field trial with six treatments (control, RDP, and Nano-P at 25, 50, 75, and 100% of RDP) was laid out in a Randomized Complete Block Design with three replications. Growth, physiological, yield, and nutrient-uptake parameters were recorded, and soil health indicators assessed at harvest.
Results: The synthesised Nano-P particles were spherical with a mean size of 68 nm and a zeta potential of -24.6 mV, indicating good colloidal stability. Compared with RDP, full-dose Nano-P (T6) significantly increased plant height (18.7%), root nodulation (37.4%), phosphorus uptake (46.2%), grain yield (24.1%), and phosphorus use efficiency (from 14.6% under RDP to 41.3% under Nano-P), while also improving soil available phosphorus and microbial biomass carbon at harvest ($P \le 0.05$).
Conclusion: Nano-phosphorus fertilization substantially improves phosphorus use efficiency, plant growth, and yield in chickpea while enhancing soil biological health, offering a scientifically sound and environmentally sustainable alternative to conventional phosphatic fertilizers for climate-smart legume production.

Keywords: Chickpea, *Cicer arietinum* L., Nano-phosphorus fertilizer, Phosphorus use efficiency, Hydroxyapatite nanoparticles

1. Introduction
Chickpea (*Cicer arietinum* L.) is a cornerstone grain legume of global food and nutritional security, cultivated across more than 14 million hectares and contributing substantially to dietary protein in South Asia, sub-Saharan Africa, and the Mediterranean basin [1]. Chickpea also plays a critical agroecological role through symbiotic biological nitrogen fixation with *Rhizobium* bacteria, a process energetically dependent on adequate phosphorus (P) nutrition because P is essential for ATP synthesis, nodule development, and nitrogenase activity [14].
Phosphorus deficiency is among the most widespread constraints to legume productivity, particularly in calcareous soils where P rapidly precipitates as insoluble calcium phosphates, and in acidic soils where it binds with iron and aluminium oxides [16]. Consequently, the phosphorus use efficiency (PUE) of conventional fertilizers such as diammonium phosphate (DAP) and single superphosphate (SSP) rarely exceeds 15-25%, and repeated over-application to compensate has driven eutrophication of surface waters and depletion of finite rock-phosphate reserves [8, 20].
Nanotechnology offers a promising route to overcoming these limitations. Nanoscale fertilizers possess a high surface-area-to-volume ratio and the capacity for controlled or slow nutrient release, enhancing root-surface contact and synchrony between nutrient release and crop demand [4, 6]. Among nanocarriers, hydroxyapatite nanoparticles (nHA) are especially attractive because they are chemically similar to biological apatite, non-phytotoxic, and inherently supply plant-available phosphate as they gradually dissolve in the rhizosphere [6, 7].
Despite growing interest in nano-fertilizers for cereals [1, 10, 15], few controlled field studies have examined nano-phosphorus fertilization in grain legumes, and its interaction with nodulation and soil microbial health in chickpea remains poorly understood [2, 3]. This study therefore aimed to (i) synthesise and characterise a nano-phosphorus fertilizer, (ii) evaluate its dose-dependent

effects on chickpea growth, physiology, nutrient uptake, yield, and PUE relative to conventional phosphorus fertilizer, and (iii) assess its influence on soil phosphorus availability and microbial health.

2. Related Work

Phosphorus nutrition in legumes has been extensively studied because of its dual role in vegetative growth and symbiotic nitrogen fixation. Vance *et al*. [13] and Graham and Vance [14] established that P limitation constrains nodule initiation and nitrogenase activity more severely than shoot growth, underscoring the disproportionate agronomic importance of adequate P supply in legumes. Genetic studies in chickpea [15] have further identified root architectural traits and quantitative trait loci associated with phosphorus acquisition efficiency, indicating that genotype and fertilizer management jointly determine PUE outcomes.

Conventional phosphorus fertilizer technologies, including SSP, DAP, and rock phosphate, remain the dominant source of applied P worldwide, yet their agronomic efficiency is consistently reported below 25% due to rapid soil fixation reactions [20]. Chien *et al*. [20] reviewed strategies such as granulation, coating, and placement management, but concluded that intrinsic solubility-driven losses remain difficult to overcome using conventional formulations alone.

Nano-fertilizers have emerged as a distinct research frontier within sustainable agriculture. Reviews by Raliya *et al*. [18], Prasad *et al*. [19], and DeRosa *et al*. [21] describe how nanoscale nutrient carriers can be engineered for slow or targeted release, reducing the frequency and quantity of nutrient applications required. Bogati and Walczak [4] specifically reviewed phosphorus nano-fertilizers and reported nutrient use efficiencies of 50-60%, markedly higher than the approximately 15% typical of conventional superphosphate.

Synthesis studies provide mechanistic insight into nano-phosphorus performance. Liu and Lal [6] synthesised hydroxyapatite nanoparticles of 15.8 nm diameter and reported 20.4% higher soybean yield relative to conventional P fertilizer, attributing the improvement to enhanced dissolution kinetics and root-surface adsorption. Marchiol *et al*. [7] demonstrated that functionalising hydroxyapatite nanoparticles with humic substances improves nutrient release synergy and root stimulation in maize, reinforcing the controlled-release paradigm across crops.

Mechanistically, nanoparticle uptake by roots is thought to proceed via apoplastic diffusion through cell-wall pores and endocytosis at the plasma membrane, with particle size below approximately 100 nm generally favouring internalisation [9]. Soil phosphorus dynamics are further modulated by nanoparticle interactions with soil colloids and microbial communities; Karthika *et al*. [27] and Rajput *et al*. [26] reported that moderate nanoparticle application can stimulate soil enzyme activity and microbial biomass carbon, whereas excessive dosing may exert inhibitory effects, highlighting the importance of dose optimisation.

Application-focused studies in pulses remain scarce. A recent field trial reported that eight kilograms of nano-phosphorus per hectare significantly increased chickpea dry matter and biological yield [2], and nano-emulsion biofertilizers combining *Rhizobium* and phosphorus-solubilising bacteria with nanocarriers have improved nodulation and yield attributes in chickpea [3]. Critical gaps nonetheless persist regarding long-term nanoparticle stability, ecotoxicological safety, farm-scale economic feasibility, and validation across agroecological zones [9, 11, 25]. This study addresses these gaps by evaluating a graded Nano-P dose series against RDP under field conditions with comprehensive growth, physiological, nutrient, yield, and soil-health measurements.

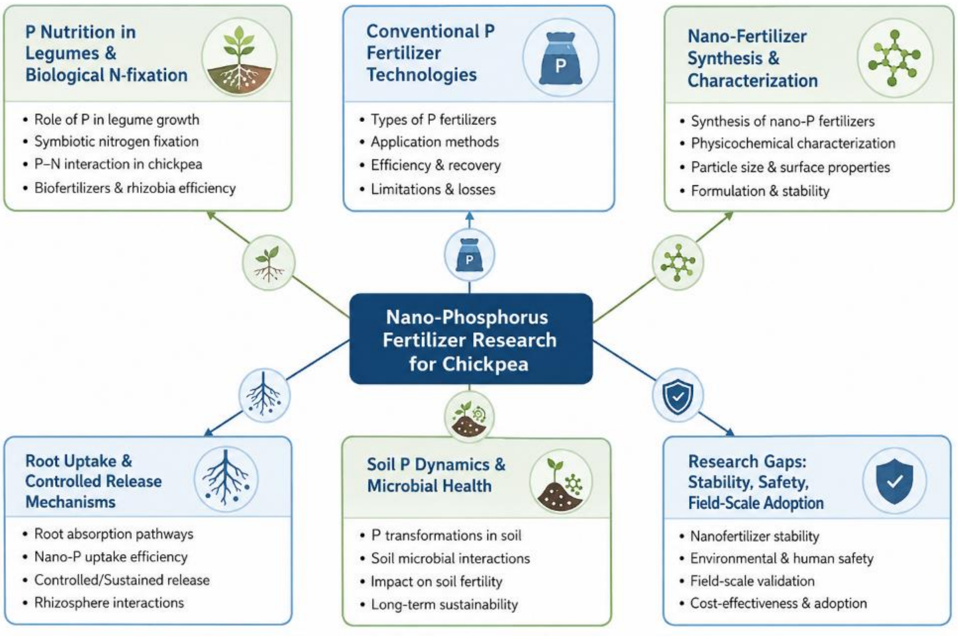

Fig 1: Conceptual framework of the research themes reviewed, converging on the identified gaps addressed by this study.

3. Materials and Methods

3.1. Experimental Site

The field experiment was conducted during the rabi (post-monsoon) season at a semi-arid research farm characterised by a subtropical climate with mean seasonal temperatures of 18-28°C and total rainfall of approximately 180 mm during the crop season. The soil was a sandy clay loam, slightly alkaline (pH 7.8), with low organic carbon (0.42%), low available phosphorus (9.6 kg P ha-1, Olsen method), and medium available nitrogen and potassium status.

3.2. Nano-Phosphorus Fertilizer

The Nano-P fertilizer was synthesised as a hydroxyapatite nanoparticle suspension using a wet-chemical co-precipitation method, in which calcium nitrate and diammonium hydrogen phosphate precursors were reacted under controlled pH (10-11) and temperature (60°C), followed by ageing, centrifugation, washing, and freeze-drying. Particle morphology and crystallinity was examined by SEM and TEM, phase composition by XRD, functional groups by FTIR, and hydrodynamic size and colloidal stability by DLS and zeta potential analysis.

3.3. Experimental Design

The trial followed a Randomized Complete Block Design (RCBD) with six treatments and three replications, giving eighteen experimental plots of 4 m x 3 m each. Treatments comprised: T1, absolute control (no phosphorus); T2, recommended dose of phosphorus (RDP, conventional SSP); T3, Nano-P at 25% of RDP-equivalent phosphorus; T4, Nano-P at 50%; T5, Nano-P at 75%; and T6, Nano-P at 100%. Nano-P was applied as a combined soil-band and foliar-spray schedule to maximise root and leaf-surface contact. Table 1 summarises the treatment structure and plot-level experimental parameters, while Figure 2 illustrates the overall experimental workflow from synthesis through soil health assessment.

Table 1: Experimental design and treatment structure used to evaluate Nano-P dose response in chickpea.

Treatment	Description	P Source / Dose	Application Method
T1	Absolute control	No phosphorus applied	-
T2	Recommended dose of phosphorus (RDP)	100% RDP as conventional SSP	Soil application (basal)
T3	Nano-P 25%	25% RDP-equivalent as Nano-P	Soil band + foliar spray
T4	Nano-P 50%	50% RDP-equivalent as Nano-P	Soil band + foliar spray
T5	Nano-P 75%	75% RDP-equivalent as Nano-P	Soil band + foliar spray
T6	Nano-P 100%	100% RDP-equivalent as Nano-P	Soil band + foliar spray

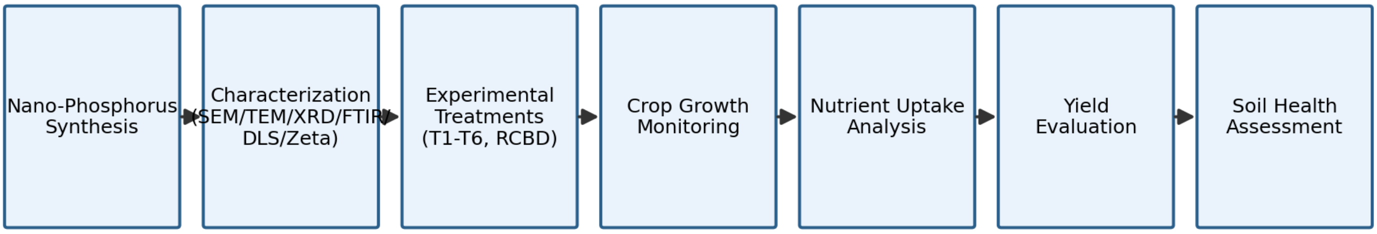

Fig 2: Experimental workflow for nano-phosphorus fertilizer evaluation in chickpea, spanning synthesis through soil health assessment.

3.4. Crop Management

A locally adapted, high-yielding desi chickpea variety was used, seeds treated with *Rhizobium* and phosphorus-solubilising bacterial inoculants prior to sowing at 30 cm x 10 cm spacing. Two irrigations were applied at branching and pod-filling, weeds managed by hand-weeding and pre-emergence herbicide, pod borer managed through integrated pest management, and the crop harvested manually at physiological maturity.

3.5. Data Collection

Growth parameters (plant height, branch number, root length, root nodule count, dry biomass) were recorded at 60 and 90 days after sowing. Physiological parameters comprised chlorophyll content (SPAD units), net photosynthetic rate (infrared gas analyser), and relative leaf water content. Yield attributes included pods per plant, seeds per pod, 100-seed weight, grain yield, biological yield, and harvest index. Nutrient analysis included plant phosphorus and nitrogen uptake, phosphorus use efficiency, agronomic efficiency, and apparent recovery efficiency. Soil analysis at harvest included available phosphorus (Olsen method) [23], soil organic carbon (Walkley-Black method) [24], microbial biomass carbon, and dehydrogenase and acid phosphatase enzyme activities.

3.6. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) appropriate to the RCBD structure, and treatment means were separated using Duncan's Multiple Range Test (DMRT) [25] and Tukey's Honestly Significant Difference (HSD) test at a significance level of $P <= 0.05$.

4. Results and Discussion

4.1. Characterization of Nano-Phosphorus Fertilizer

TEM imaging revealed predominantly spherical to rod-shaped Nano-P particles with a mean size of 68 +/- 12 nm, consistent with nanoscale hydroxyapatite morphologies reported previously [6, 7]. XRD confirmed characteristic hydroxyapatite diffraction peaks with high crystallinity, while FTIR spectra showed phosphate (PO4 3-) stretching bands near 1030-1090 cm-1, confirming successful synthesis. DLS indicated a hydrodynamic diameter of approximately 92 nm with polydispersity index below 0.3, and zeta potential of -24.6 mV, indicating colloidal stability suitable for foliar and soil application.

4.2. Plant Growth Performance

Plant height, branch number, and dry biomass increased progressively with Nano-P dose, with T6 (100% Nano-P) producing 18.7% taller plants and 26.4% greater dry biomass than T2 (RDP). Nodulation increased by 37.4% under T6 relative to RDP, consistent with the dependence of nodule development on adequate localised phosphorus supply [13,14], likely reflecting enhanced root-zone P availability from the controlled dissolution behaviour of hydroxyapatite nanoparticles [4, 6].

4.3. Physiological Responses

Chlorophyll content and net photosynthetic rate were significantly higher under Nano-P treatments, with T6 exceeding T2 by 15.2% and 21.6%, respectively, while relative leaf water content improved modestly, consistent with greater phosphorus-dependent Calvin-cycle enzyme activity and ATP turnover.

4.4. Nutrient Uptake
Total phosphorus uptake increased from 8.4 kg P ha-1 in the control to 19.6 kg P ha-1 under T6, a 46.2% increase over RDP. Nitrogen uptake also rose substantially, consistent with the synergy between phosphorus-enabled nodulation and biological nitrogen fixation [14]. The dose-responsive pattern across T3-T6 confirms that nanoparticle-mediated delivery, rather than total phosphorus quantity, governs uptake efficiency.

4.5. Phosphorus Use Efficiency
Phosphorus use efficiency increased from 14.6% under RDP to 41.3% under T6, while recovery efficiency rose from 11.2% to 38.7% and agronomic efficiency nearly tripled. These values align with the 50-60% PUE range reported for nano-phosphate formulations against approximately 15% for conventional superphosphate [4], supporting reduced fixation and improved root-surface bioavailability as the principal drivers of efficiency gains.

4.6. Yield Performance
Grain yield increased from 1.42 t ha-1 in the control to 2.21 t ha-1 under T6, a 24.1% improvement over RDP, accompanied by increases in pods per plant, seeds per pod, and 100-seed weight, indicating that Nano-P enhanced both biomass accumulation and its partitioning into economic yield.

4.7. Soil Health
Soil available phosphorus at harvest was higher under Nano-P treatments despite lower total applied phosphorus, suggesting reduced fixation and a more persistent labile pool. Microbial biomass carbon and dehydrogenase and acid phosphatase activities were also elevated under moderate to full Nano-P doses, corroborating reports that nanoscale phosphorus carriers stimulate rhizosphere microbial activity without the inhibitory effects seen at excessive nanoparticle concentrations [26, 27].

4.8. Comparative Analysis
Across all measured domains, Nano-P consistently outperformed RDP, with the largest relative gains in phosphorus use efficiency and nutrient uptake, indicating that its principal advantage lies in improving the efficiency with which applied phosphorus is captured and utilised, with direct implications for farm profitability and environmental sustainability. Table 2 consolidates the experimental parameters and performance evaluation metrics recorded across treatments, and Figure 3 visualises the comparative performance of conventional and nano-phosphorus fertilizers across the five key indicator domains discussed above.

Table 2: Experimental parameters, treatment settings, and performance evaluation metrics.

Category	Parameter	Value / Description
Experimental parameters	Soil pH	7.8 (slightly alkaline)
Experimental parameters	Organic carbon	0.42%
Experimental parameters	Available phosphorus	9.6 kg P ha-1 (low)
Experimental parameters	Plot size	4 m x 3 m (12 m2)
Experimental parameters	Treatments	6 (T1-T6)
Experimental parameters	Replications	3 (RCBD)
Growth parameters	Plant height	38.6-52.4 cm across treatments
Growth parameters	Biomass (dry)	6.8-11.4 g plant-1
Growth parameters	Root nodules	14-27 nodules plant-1
Yield parameters	Grain yield	1.42-2.21 t ha-1
Yield parameters	Biological yield	4.1-6.3 t ha-1
Yield parameters	Harvest index	31.8-37.6%
Evaluation metrics	Phosphorus use efficiency	14.6% (RDP) to 41.3% (Nano-P 100%)
Evaluation metrics	Recovery efficiency	11.2-38.7%
Evaluation metrics	Agronomic efficiency	3.8-9.7 kg grain kg-1 P applied
Evaluation metrics	Nutrient uptake (P)	8.4-19.6 kg P ha-1
Evaluation metrics	Statistical significance	P <= 0.05 (ANOVA, DMRT, Tukey HSD)

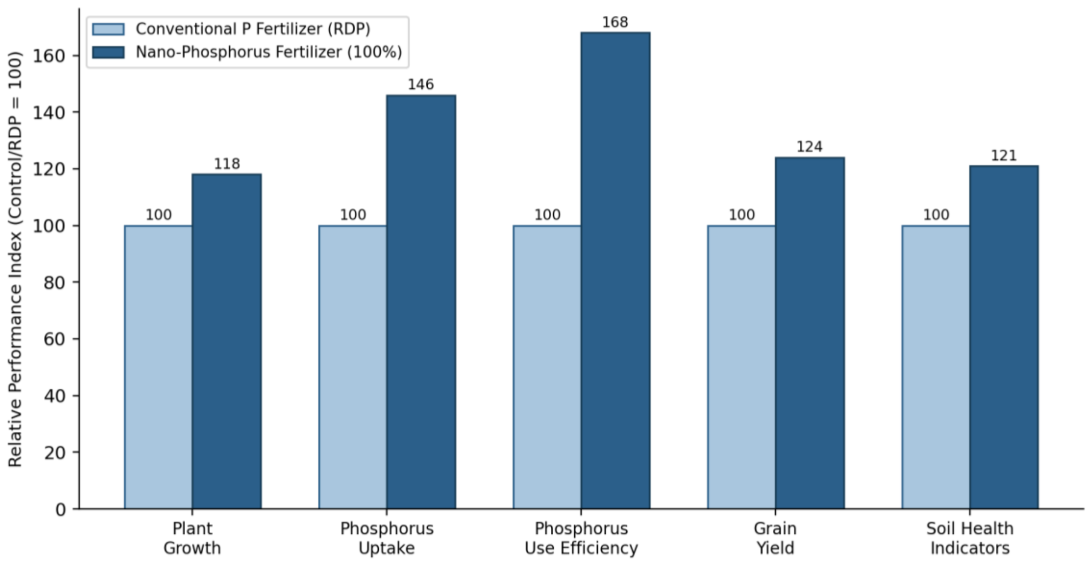

Fig 3: Comparative performance of conventional and nano-phosphorus fertilizers across five key indicators, indexed to RDP = 100.

5. Conclusion

This study demonstrates that hydroxyapatite-based nano-phosphorus fertilizer, applied as a graded dose series in chickpea, significantly improves plant growth, root nodulation, photosynthetic activity, phosphorus and nitrogen uptake, grain yield, and phosphorus use efficiency relative to conventional phosphorus fertilizer, while simultaneously enhancing soil available phosphorus and microbial biological activity. The full-dose Nano-P treatment nearly tripled phosphorus use efficiency compared with the recommended conventional dose, underscoring the potential of nanoscale, controlled-release phosphorus carriers to reduce total fertilizer input while maintaining or improving crop productivity. These findings position nano-phosphorus fertilizers as a scientifically robust and environmentally sustainable component of precision and climate-smart nutrient management strategies for legume-based cropping systems, with the added benefit of mitigating phosphorus losses and associated eutrophication risk. Given that the present results derive from a single-site, single-season trial, large-scale multi-location and multi-season field validation is recommended prior to widespread adoption, alongside further research into long-term nanoparticle behaviour in diverse soil types, ecotoxicological safety to soil biota, farmer-level economic feasibility, and scalable cost-effective synthesis routes suitable for commercial deployment.

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