

Fe and Zn Metal Nanocitrates as Plant Nutrients through Soil Application

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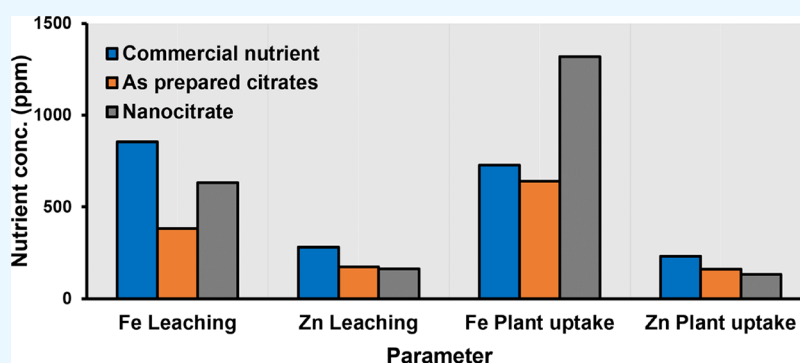
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ABSTRACT: Nanocitrates of iron (Fe) and zinc (Zn) in the form of plant nanonutrients were examined for their behavior in soil and the uptake of these by 20-day old groundnut (*Arachis hypogaea*) seedlings under greenhouse conditions. The Fe (0.04 to 0.008 mmol/kg of soil) and Zn (0.02 to 0.004 mmol/kg of soil) nanocitrates were applied to soil and compared with commercial counterparts (FeSO_4 , ZnSO_4 , nano-Fe, nano-Zn, Fe-EDTA, Zn-EDTA). The combined nanocitrate compositions were also formulated by physical means and characterized. The plant uptake of Fe and Zn was determined through atomic absorption spectrometry (AAS). All the treated plants showed good germination and higher vigor indexes compared to the control treatments. The highest available Fe and Zn soil contents after leaching were 150.5 and 18.9 mg/kg, respectively, in combined nanocitrate compositions, whereas in the control (untreated) soil, the Fe and Zn contents were 6.0 and 0.7 mg/kg, respectively. The plant's Fe content was 0.48 mg/pot for the combined nanocitrate composition, and that of the untreated plant sample was 0.02 mg/pot. The plant's Zn content was 82.3 μg /pot for pure zinc citrate, and the respective untreated-plant Zn content was 2.1 μg /pot. These values are better than those observed for commercial fertilizers. Additionally, no trend in promotional and antagonistic correlations between Fe and Zn in combined nanocitrates was observed in the studied period (20 days in duration). Among the 34 synthesized citrates, six nanocitrates show promising trends for evaluation under field conditions with higher stability.

INTRODUCTION

The enhancement of crop productivity and quality is of paramount importance in modern agriculture to achieve a target of 300 million tons of food by 2025 to feed an increasing population. Crop nutrition is an integral component of modern agriculture, and fertilizers comprise a major agro-input in this context. India requires 45 million tons of nutrients against the current consumption of 23 million tons. Unfortunately, the productivity of crops suffers due to nutritional deficiencies in the soil. Due to nutritional deficiencies, the quality of produce and the overall yield of crops will be reduced. The extent of multi-nutrient deficiencies is increasing day by day, leading to crop losses of nearly 25–30%.¹ To correct these nutritional deficiencies, farmers are applying large commercial fertilizers, leading to disturbance in the soil mineral balance and fertility and also creating environmental concerns. However, the real potential of fertilizers being applied remains unutilized

primarily due to bottlenecks of leaching, runoff, fixation, and reactivity in the soil, leading to lower plant use efficiency.

Micronutrients have a unique role in plant growth and development and, in many cases, represent a limiting factor for maximum production.^{2–4} Various application methods of micronutrients in plants through broadcast, foliar spray, and pre-sowing seed treatment methods are being followed.^{5–7} Furthermore, applying micronutrients through the soil and seed treatment could assist plants through early critical growth stages toward yield improvement.^{8,9} However, among those micronutrients, iron is special in many aspects due to its

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sensitivity to many climatic and edaphic conditions that facilitate its fixation or unavailability.^{10,11} Iron is one of the essential elements for plant growth and is crucial in photosynthetic reactions, activating numerous enzymes involved in the transfer of energy, reduction and fixation of nitrogen, the formation of lignin, and its contribution to ribonucleic acid synthesis.¹² Zinc is the second most abundant transition metal in organisms after iron. Also, Zn is the only metal represented in all six enzyme classes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases.¹³ Furthermore, several reactions are catalyzed by iron and zinc compounds in plants. Iron and zinc are essential micronutrients for humans, animals, and plants. According to Graham et al.,¹⁴ throughout the world, more than 3 billion people suffer from micronutrient deficiencies. As a result, there is an increase in the research toward developing technologies for increased uptake and accumulation of micronutrients in comestible plant parts.

Nanoparticles (NPs) interact at the molecular level in living cells, and employing nanoparticles in agriculture could impart some beneficial effects in seed germination and the control of plant diseases.¹ Using NPs and nanopowders as carriers, controlled or delayed-release fertilizers have been developed.^{15,16} NPs are highly reactive due to their specific surface area, high-density reactive areas, or increased reactivity on the particle surfaces. Additionally, the same features facilitate easy absorption of pesticides and fertilizers produced.¹⁷ Thus, nanotechnology can revolutionize agriculture with new tools to enhance the ability of plants to absorb nutrients.

The benefit of nanotechnology is the fact that nanofertilizers and other nanomaterial-based designs have much more potential to boost agriculture.^{18–21} In the recent past, using nanoparticles (NPs) in agriculture has become more popular, and NPs as fertilizers are very effective.^{22–25} Thus, NPs can be used to control the release of concerning nutrients, and it would be a helpful strategy to resolve the issue of low agronomic productivity.²⁶ Because of the nanosize of the particles, the plants can absorb them through different routes compared to bulk particles, which present an added advantage. Several inorganic, organic, and composite nanomaterials have been evaluated for their impact on plant growth and productivity of various crops. Some of the nanoparticles are used as nutrients/fertilizers and validated on agriculturally important crops. Among various other NPs, zinc oxide nanoparticles improved plant growth while abating oxidative damage in cotton.^{27–29} Applying iron nanoparticles improved plant development and reduced metal uptake in wheat plants under stress.³⁰ The mechanisms underlying NP-mediated nutrient uptake vary with the NP type, mode of application, time of NP exposure, and plant species. In this way, NPs can enhance plant improvement and nutrient uptake by minimizing nutrient losses or enhancing the plant defense system.^{28,29,31}

Numerous studies have reported applying a single type of NP under biotic and abiotic stresses in various crops. Many of the studies have been conducted in pot experiments. Little information is known about NPs' role in leaching and sedimentation studies under laboratory conditions. It was hypothesized that the combined application of various NPs could have positively affected plants. Both Fe and Zn are chosen due to their importance as plant nutrients and their deficiency in the Earth's soils. Metal NPs, including nanoscale Fe and Zn, may interact with SOD (superoxidase dismutase)

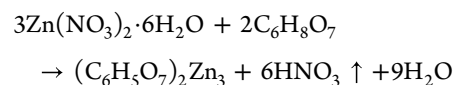
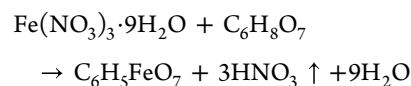
and modify their biochemistry and functioning since Fe and Zn are structural constituents of SODs.³² The combined Fe-SOD and Zn-SOD seem to be the groups following the metal co-factor used by the plant.³³

Chelation-based micronutrients find their prominence due to protection from fixation and excess washout. Among available chelators, EDTA and EDDHA are carcinogenic and toxic to human beings.³⁴ To avoid such unwanted risks, we have chosen greener chelators such as citric acid for our study. In this study, we also formulated the combination of Fe and Zn nanocitrates and examined their effects on the plants. The impact of single and combined Fe and Zn nanocitrates on groundnut plant growth and nutrient uptake through soil application was studied. The selection of the best combinations that performed well from various parameters was done. The goal of this study is to discuss the applicability of metal nanocitrates as a plant nutrient, and the fates of nanocitrates and other Fe and Zn commercial fertilizers were comparatively studied in soil and on 20-day old groundnut seedlings. Among the studied commercial nutrients, the highest Fe content of 0.19 mg/pot was observed in nano-Fe and the highest Zn of 88.5 $\mu\text{g}/\text{pot}$ in nano-Zn. The Fe content was observed to be significantly high in nanocitrates, and the Zn content was on par with nanocitrates compared to studied commercial Fe and Zn nutrients. From the study, we show that nanocitrates performed better than commercial nutrients.

MATERIAL AND METHODS

Ferric nitrate hydrous (FN; $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$), purity of 98%, zinc nitrate hydrous (ZN; $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), purity of 98%, and citric acid anhydrous (CA; $\text{C}_6\text{H}_8\text{O}_7$), purity of 99%, were purchased commercially from SRL, India. All the reagents were used as such without further purification.

Synthesis of Combined Iron (Fe) and Zinc (Zn)-Based Citrates and Their Nanocitrates. In our earlier reports, the ferric citrate and zinc citrate were prepared by a solid-state grinding technique and their nanocitrates by further ball-milling.³⁵ The samples were coded as FC (1:1) and ZC (1:3) where the ratio indicates the respective moles. The ball-milled samples were coded as BFC (1:1) and BZC (1:3). Similarly, the combined citrates of iron and zinc were prepared by mixing ferric citrate (FC) and zinc citrate (ZC) of different weight ratios. The total weight was maintained as 10 g. The resulting mixture was grounded for 45 min.



The ground citrates were dried at 80 °C in a hot air oven. The prepared citrates were further used in the studies without any further purification. The samples were coded as FZ (x:y) where x and y are the weights of FC and ZC used for the synthesis, respectively. These samples are referred to as prepared samples in the paper.

The combined ferric and zinc nanocitrates were prepared through planetary ball-milling (XQM-1-A, Changsha Tianchuang Powder Technology Co., Ltd., China) attached with a cooling unit to provide temperatures of 2–10 °C of FZ (x:y) for different durations. The differently ball-milled samples were

Table 1. Details of Various Samples of Iron (Fe) and Zinc (Zn) Used for Study

as-prepared citrates	ball-milled citrates				commercial micronutrients
FC (1:1)	BFC (1:1)–6	BFZ (5:5)–2	BFZ (2:8)–6	BFZ (6:4)–10	FeSO ₄
ZC (1:3)	BZC (1:3)–6	BFZ (5:5)–4	BFZ (2:8)–8	BFZ (4:6)–2	ZnSO ₄
FZ (8:2)	BFZ (8:2)–2	BFZ (5:5)–6	BFZ (2:8)–10	BFZ (4:6)–4	nano-Fe - Geolife
FZ (6:4)	BFZ (8:2)–4	BFZ (5:5)–8	BFZ (6:4)–2	BFZ (4:6)–6	nano-Zn - Geolife
FZ (5:5)	BFZ (8:2)–6	BFZ (5:5)–10	BFZ (6:4)–4	BFZ (4:6)–8	Fe-EDTA - Fe Gro
FZ (4:6)	BFZ (8:2)–8	BFZ (2:8)–2	BFZ (6:4)–6	BFZ (4:6)–10	Zn-EDTA - ZinGap
FZ (2:8)	BFZ (8:2)–10	BFZ (2:8)–4	BFZ (6:4)–8		control (untreated)

used as such for further studies. The ball-milled samples were coded as BFZ (*x*:*y*)–*Z* (*Z* = 2, 4, 6, 8, and 10 h).

Characterization of Combined Nanocitrates. The X-ray fluorescence spectroscopy (XRF) was conducted using Panalytical Epsilon-1 with an Omnic Fast Mode using Epsilon 3 software, directly measuring the weighed amount of samples for Fe and Zn content measurements. The weight percentage was within $\pm 0.5\%$. The as-prepared and nanocitrates of Fe and Zn were characterized for their functional groups and particle size after completely drying. FTIR spectra were recorded using a Thermo Nicolet (is50) spectrometer using a diamond ATR crystal. Transmission electron micrographs (TEM) were taken on a Tecnai T20, 200 keV, FEI. The dried grids were used for TEM imaging. For dynamic light scattering instrument (DLS) characterization, the samples were prepared by dispersing in pure isopropanol. The dispersed samples were loaded on a Cu grid and dried at 60 °C. The hydrodynamic size and zeta potential measurements were collected using a Malvern Zeta-Sizer NanoZS (Malvern Instruments, USA). The samples were equilibrated for 2 min at 25 °C before obtaining each measurement. For both TEM and DLS, the samples were dispersed at 0.01 g/mL in isopropanol.

Evaluation of Combined Fe and Zn Citrates. The as-prepared and ball-milled combined Fe and Zn citrates were used to evaluate their effect on the plant uptake, plant total dry matter, and soil availability and the effect of nutrient leaching from soil; the performance was compared with that of commercial Fe and Zn samples. A total of 41 samples were chosen for the entire study, which consists of 7 as-prepared citrates, 27 ball milled citrates, and 6 commercial Fe and Zn micronutrients, and the details are given in Table 1. An untreated sample (without the addition of any nutrient) was also taken as a control sample. Each sample was applied to red soil in pots as per the rates given in Table 2. The powder forms of samples were mixed in the red soil uniformly. The entire experiment was conducted in a completely randomized design with three replicates.

Sedimentation Studies. The sedimentation studies on the samples were performed as per the procedure reported in the earlier literature.³⁶ The samples were added to dry soil (400 mg/kg). The soil extracts were prepared by mixing the soil with water in a weight ratio of 1:5 and shaking for 5–6 h at 150 rpm. The soil suspensions were bath-sonicated for 5 min immediately before use in static sedimentation studies over 30 days. The sedimentation dynamics was measured using a multi-mode microplate reader spectrophotometer (BioTek, USA). The multi-mode spectrophotometer was calibrated using nanopure water. The absorbance was measured at 600 nm. The sedimentation readings were taken at different weekly intervals. The patterns were inferred from the time course of a normalized suspension absorbance at 600 nm (A/A_0 where A_0 was the initial absorbance at the start of the experiment). The

Table 2. Fe and Zn Contents Present in Studied Samples

sample	Fe content/kg of soil	Zn content/kg of soil
FC (1:1)	0.040 mmol	
ZC (1:3)		0.020 mmol
FZ (8:2)	0.032 mmol	0.004 mmol
FZ (6:4)	0.024 mmol	0.008 mmol
FZ (5:5)	0.020 mmol	0.010 mmol
FZ (4:6)	0.016 mmol	0.012 mmol
FZ (2:8)	0.008 mmol	0.020 mmol
FeSO ₄	0.016 mmol	
ZnSO ₄		0.015 mmol
nano-Fe	0.025 mmol	
nano-Zn		0.025 mmol
Fe-EDTA	0.025 mmol	
Zn-EDTA		0.025 mmol

absorbance of the filtered soil extract alone was also checked over time as a control experiment.

Incubation Studies. About 500 g (oven dry weight) of soil (loamy sand alfisols having a neutral pH with more than 0.75% of organic carbon content) were mixed separately with 2 g of citrate and commercial samples and filled in plastic containers (8 cm diameter \times 15 cm length). Before use, the soil was completely analyzed for its physicochemical properties, which are presented in Table 3. The plastic containers were provided

Table 3. Physicochemical Properties of Soil Used for Study

soil parameters	red soil
pH	6.5
electrical conductivity (dS/m)	0.27
bulk density (g/cm ³)	1.41
organic carbon (%)	0.75
clay (%)	10.5
sand (%)	83.25
silt (%)	6.25
available nitrogen (kg/ha)	312
available phosphorus (kg/ha)	27
available potassium (kg/ha)	292
available sulfur (mg/kg)	8.2
available Fe (mg/kg)	5.5
available Zn (mg/kg)	1.0

with a basal 1 cm glass wool pad over the drainage pores.³⁷ The measurements were made in triplicate. Initially, the red soil after treatment with nutrients was allowed for incubation. The incubation was maintained for 4 days at field capacity with deionized water. A volume of 25 mL of deionized water was added to the incubated soils, and the leachates were collected. The Fe and Zn contents were determined using an atomic absorption spectrophotometer (AAS). The Fe and Zn in soil were also analyzed before and after the leaching of nutrients.

DTPA Extraction of Fe and Zn. An amount of 10 g of soil was collected and air-dried. The extraction of the air-dried soil was done with 20 mL of DTPA (diethylenetriamine pentaacetate) for determining the available micronutrients in the soil.³⁷ The supernatant collected from the soil sample was filtered through a Whatman 42 filter paper and used for AAS analysis.

Evaluation of Combined Fe and Zn Citrates on the Plant Growth of Groundnut. Groundnut (*Arachis hypogaea* var. kadirri 6) seeds were obtained from the seed unit section of the ICAR–Indian Institute of Oilseeds Research, Hyderabad. The experiment on plants was conducted as per institutional and national guidelines and legislation. Seeds were sown in small pots containing 1 kg of 2 mm sieved red soil. The same soil was used for incubation, leaching, and plant growth and uptake studies. About 15 surface sterilized groundnut seeds were sown in each pot and were maintained in a seed incubator having a temperature of 27 ± 1 °C for 20 days. No visual symptoms of deficiencies or toxicity of Fe and Zn were observed. The experiment was conducted in triplicate using a completely randomized design. The seedlings were watered with an equal amount of deionized water based on the requirement in all treatments. On the 21st day, the number of germinated seedlings was counted; we rinsed them thoroughly with deionized water and took the shoot and root length of each seedling. For dry-weight determination, the seedlings were dried at 60–70 °C for 48 h.³¹

Atomic Absorption Spectrophotometer (AAS) Analysis. The dried plant samples were grounded in a pestle and mortar for mineral analysis until the dry powder was obtained. After that, the sample (0.5 g) was digested using diacid followed by dilution to a known concentration, which was used for analysis of Fe and Zn contents using AAS.³⁵ The digested and diluted samples were used immediately for quantification of Fe and Zn, and all the analyses were carried out in triplicate.

The atomic absorption spectrometer (Perkin Elmer PinAAcle 900F, USA) was used to determine Fe and Zn with an air–acetylene flame. For Fe, the instrumental parameters employed were as follows: wavelength of 248.3 nm, lamp current of 12 mA, bandpass of 0.2 nm, and a hollow cathode lamp for the background correction. For Zn, the following instrumental parameters were set: wavelength of 213 nm, lamp current of 12 mA, bandpass of 0.2 nm, and a hollow cathode lamp for the background correction. The calibration graph was obtained by feeding working standards of 0.5, 1.0, 2.0, and 3.0 mg/L for Fe and 0.2, 0.4, 0.6, and 0.8 mg/L for Zn. Working standards for calibration were obtained by appropriate dilution of stock solutions (1000 mg/L) of Fe and Zn (Merck Pure AAS Standards) using deionized water. The AAS method used to estimate Fe and Zn was corrected for deionized water and the acid used for digestion. WINLAB 32 AA version 7.4.1 software was used for the final quantification and had several quality control check options. A two-coefficient equation was used, and a minimum correlation coefficient of 0.995 was assigned in the software for QC check. An S-shaped calibration curve was detected for standards and then proceeded for nutrient Fe and Zn quantification.

Statistical Data Analysis. Analytical determination was carried out from the average mean data of 45 plants in each set for all treatments. All experimental data were expressed as the mean of replicates and analyzed using analysis of variance (ANOVA) using R version 4.0.4 software.

The contribution of each character to the total variation of all samples was determined by principal component analysis (PCA). The principal components (PCs) were evaluated using R version 4.0.4 software. It is difficult to conclude the best performer for all the characters by considering the performance of treatment for each character separately. The best performers were identified by constructing a combined weighted index for all the studied characters. We have developed a weighted index approach based on the principal components in the present study. In this method, weights will be given for these physiological characters based on the principle that the variation in the linear combination of these characters should be the maximum. The weighted index can be defined as

$$I = \frac{\sum_{i=1}^n X_i \left(\sum_{j=1}^n |L_{ij}| \cdot E_j \right)}{\sum_{i=1}^n \left(\sum_{j=1}^n |L_{ij}| \cdot E_j \right)}$$

where I is the index, X_i is the i th indicator, L_{ij} is the factor loading value of the i th variable on the j th factor, and E_j is the eigenvalue of the j th factor.

RESULTS AND DISCUSSION

Fe and Zn Content in Citrates. The amounts of Fe and Zn in the synthesized combined and individual citrates were estimated using XRF. The Fe content ranged from 10.6 to 21.5%, whereas the Zn content ranged between 14.2 to 29.8%. The value range finds potential good sources of Fe and Zn (Table 4) as plant nutrients in citrates. The Fe and Zn contents available within citrates are in the range of market available micronutrients and nanonutrients.

Table 4. Fe and Zn Contents in Citrates

sample	Fe content (%)	Zn content (%)
FC (1:1)	20.7	
ZC (1:3)		29.8
FZ (8:2)	21.5	14.2
FZ (6:4)	20.2	22.1
FZ (5:5)	20.9	23.2
FZ (4:6)	18.7	26.2
FZ (2:8)	10.6	27.8

FTIR, TEM, and DLS Analyses of Combined Citrates. The frequency assignment of IR spectra for the combined citrates collected after completely drying is represented in Figure 1, and more details are in Table S1. The characteristic strong doublet peak at 1700 and 1620 cm^{-1} in all the metal citrate samples is attributed to carboxylate groups that confirm the coordination of Fe^{3+} and Zn^{2+} to the citrates. No differences were observed in the FTIR spectra of corresponding ball-milled citrates.

Figure 2A,B shows the TEM images of as-prepared and ball-milled combined citrates and a histogram of the particle size distribution obtained from TEM images. The sizes of nanocitrates from TEM images were collected using imageJ software, and sizes are in the range of 1.5 to 5.6 nm for 10 h ball-milled citrates. The TEM images of combined citrates indicate that the samples are aggregated and have a spherical morphology. The particle size decreased due to ball milling, and aggregation was observed in all images. The aggregation of metal citrates is due to the hygroscopic nature of the citrates. With the increase in the ball-milling duration, the aggregation

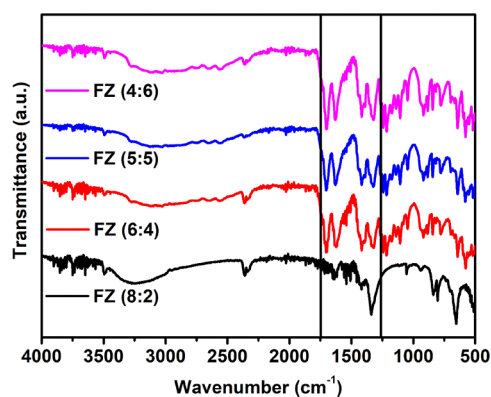


Figure 1. FTIR spectra of combined citrates.

of metal citrates was reduced, which is evidenced by TEM images.

The hydrodynamic diameters of the combined citrates range from 204.7 to 1455 nm, depending on the Fe:Zn ratio and ball-milling duration. The zeta potentials were determined majorly

to be negative except for two samples where they are positive. The zeta potential values range between -8.3 and 0.3 mV, which are given in Table S2.

Sedimentation studies were performed to determine the stability of the nanoparticles in soil extracts (Figure 3). A higher sedimentation rate was observed immediately after seven days of incubation, which may be due to the aggregation of particles. The sedimentation rate was reduced on the 15th day and 30th day. There was not much difference between sedimentation rates during the 15th and 30th day for all the samples; however, there were few exceptions. For example, in BFZ (4:6)–6, the sedimentation rate increased from 0.14 to 0.26. The sedimentation rate varied from 15 to 30 days in Fe-EDTA (0.22 to 0.12) and BFZ (4,6)–10 (0.15 to 0.08).

Changes in DTPA Extractable Fe and Zn Contents during Incubation. DTPA extractable Fe and Zn contents in soil significantly varied with samples. The maximum DTPA-Fe content was recorded before incubation, that is, 98.58 mg/kg in Fe-EDTA (commercial), whereas the highest Fe content in citrates was 65.81 mg/kg in the BFZ (4:6)–8 sample (149.3 mg/kg after incubation). There was a reduction of DTPA-Fe

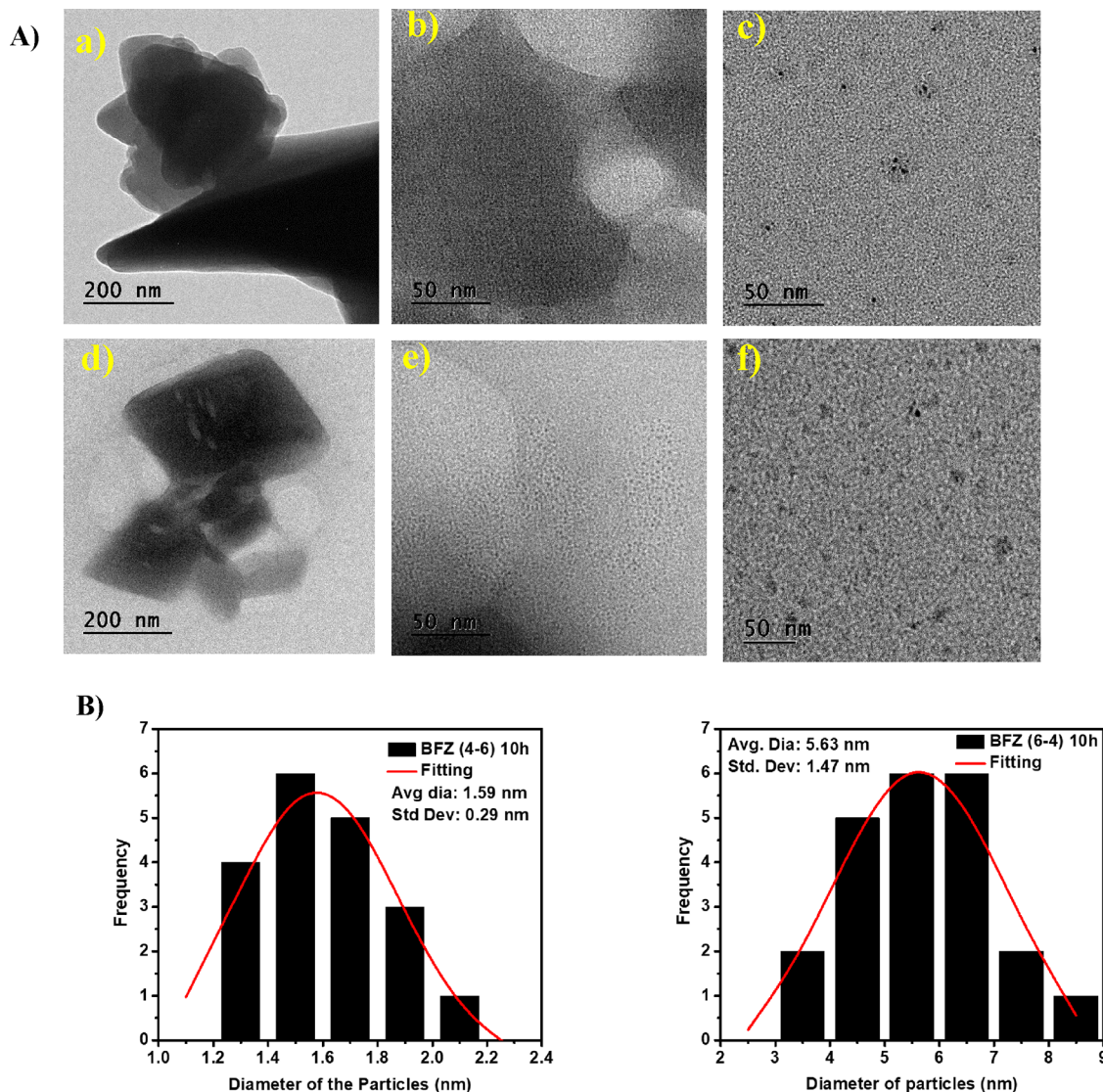


Figure 2. (A) TEM images of (a) FZ (4:6)–0, (b) BFZ (4:6)–6, (c) BFZ (4:6)–10, (d) FZ (6:4)–0, (e) BFZ (6:4)–6, and (f) BFZ (6:4)–10. (B) Histogram containing average particle sizes of BFZ (4:6)–10 and BFZ (6:4)–10 samples.

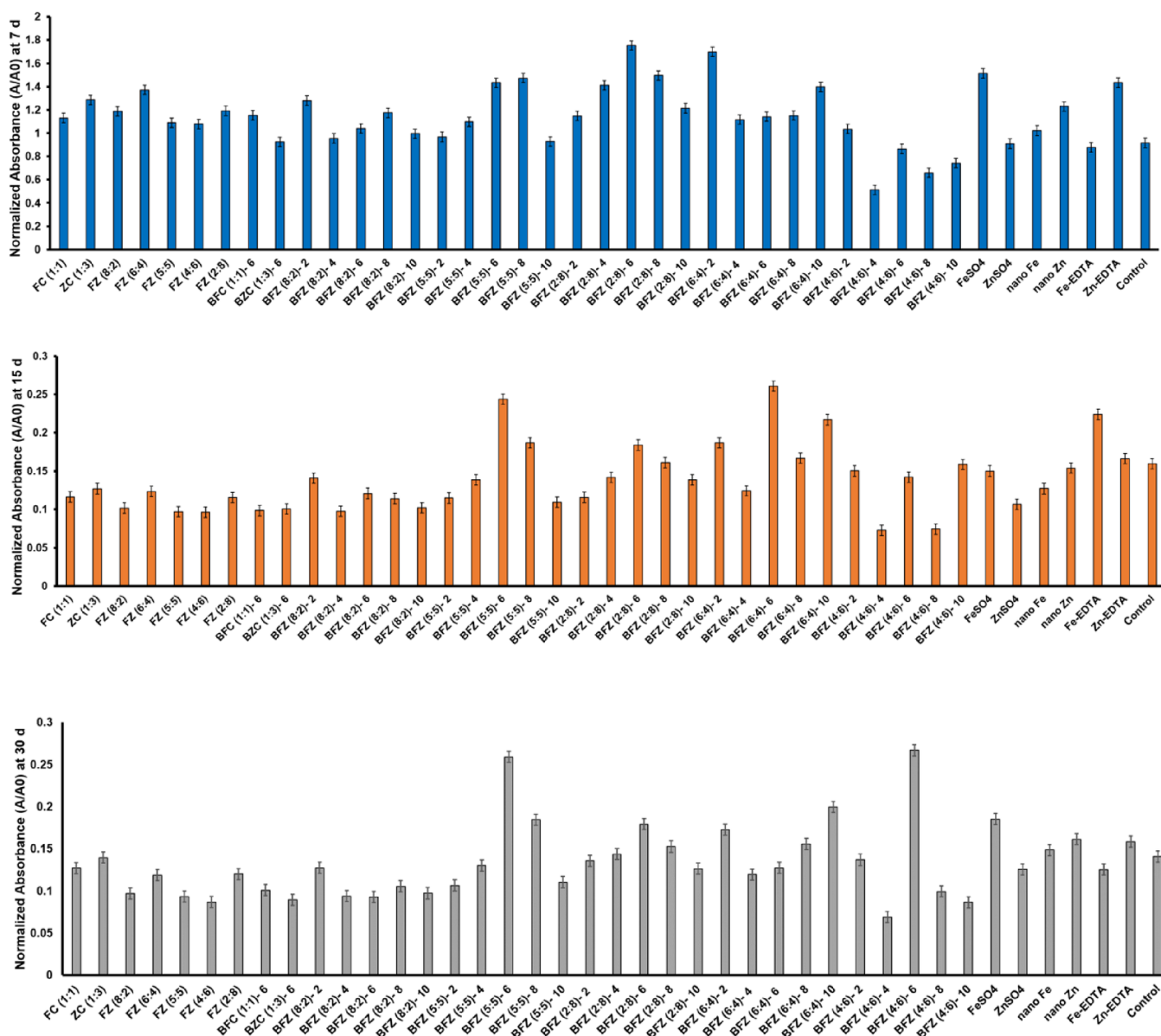


Figure 3. Sedimentation of Fe and Zn citrates in the soil extract at four intervals for 7, 15, and 30 days. The critical difference (CD) at 5% degree of freedom is 0.43; the coefficient of variation (CV) is 1.09.

content at the end of the incubation period followed by 35.23 mg/kg of leaching in Fe-EDTA (commercial) as given in Table S3. In most of the citrates, the Fe content increased after incubation followed by leaching in all samples except for a few samples such as FC (1:1) (reduced from 53.5 to 39.1 mg/kg), BFC (1:1)–6 (reduced from 46.5 to 20.1 mg/kg), and BFZ (4:6)–2 (reduced from 62.7 to 60.6 mg/kg). The highest DTPA-Zn was recorded in nano-Zn (210.6 mg/kg) followed by ZnSO₄ (117.9 mg/kg) and BZC (1:3)–6 (66.2 mg/kg) as given in Table S3.

Leaching Effect. Leaching studies indicate the solubility and mobility of nutrients in the soil. The leaching studies give an idea about nutrient availability at very different rooting depths. Soluble Fe and Zn contents of nitrates, sulfates, and commercial nanonutrients varied significantly. In citrate samples, less Fe leaching of below 10 ppm was observed in the BFZ (8:2)–8 and BFZ (4:6)–8 samples, and Zn leaching of ~2 ppm was observed in the samples such as BFZ (8:2)–2, nano-Zn, BFZ (5:5)–4, BFZ (8:2)–4, BFZ (2:8)–6, BFZ

(6:4)–2, and BFZ (2:8)–8. The highest soluble Fe content was observed in samples such as Fe-EDTA (855.3 mg/L), FZ (6:4) (382.6 mg/L), FeSO₄ (134.9 mg/L), and nano-Fe (159.5 mg/L), whereas in the case of Zn, the highest soluble content was observed in Zn-EDTA (280.4 mg/L), ZC (1:3)–0 (172.77 mg/L), ZnSO₄ (141.63 mg/L), and nano-Zn (164.41 mg/L) as shown in Table S3. On average, the amount of soluble Fe content varied from 3.87 to 855.33 mg/L, and the soluble Zn content varied from 0.34 to 280.43 mg/L.

Evaluation of the Effect of Combined Fe and Zn Citrates on the Plant Growth of Groundnut. Groundnut seeds responded variably toward treatment with various Fe and Zn citrates along with commercial samples. The highest significant germination (80%) was observed in samples such as FZ (8:2)–0, BFC (1:1)–6, BZC (1:3)–6, BFZ (2:8)–2, BFZ (4:6)–2, and nano-Zn, and the highest seedling vigor index in the BZC (1:3)–6 (3012.84) sample. The seedling quality of all samples including that of the commercials and the untreated ones is presented in Table S3. The germination was 40%, and

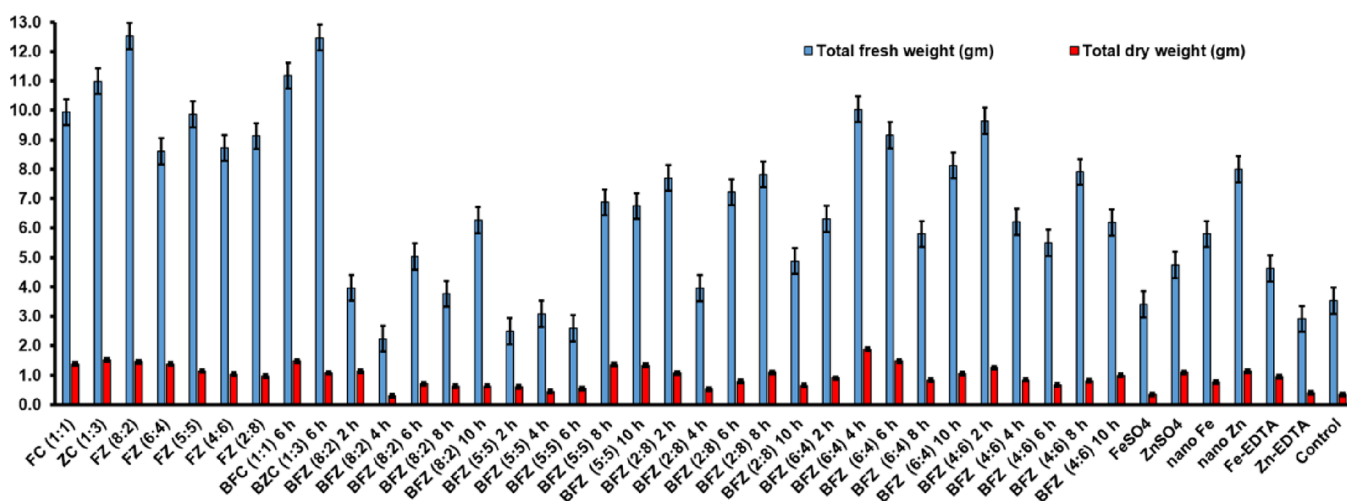


Figure 4. Total fresh weights and total dry weights (gm) of groundnut seedlings after treating with Fe and Zn nutrients.

the vigor index was 727.33 for untreated groundnut seedlings. However, although we have observed improvement in the germination and vigor index of almost all samples, few samples performed even less than the values of control experiments.

The effect of Fe and Zn citrate nanoparticles and as-prepared citrate particles on the root and shoot length of groundnut is presented in Table S3. The root length of citrate-treated groundnut is drastically less in almost all samples with the least root-to-shoot ratio observed in BFZ (8:2)–4 (0.34). A few samples, namely, BFZ (4:6)–4, BFZ (6:4)–10, BFCZ (1:1:1)–10, FeSO₄, and BFZ (5:5)–2, exhibited a higher root-to-shoot ratio. The highest root-to-shoot ratio in BFZ (5:5)–2 nanoparticle-treated plants was only 1.14, but it is more than that of plants in the control experiment (0.76). The root-to-shoot ratio decreased with ball-milling duration time between 4 and 6 h, and then it increased further up to 10 h (Table S3).

An increase in the fresh and dry weights of treated plants was observed in all samples compared to control (untreated) experiments except for very few samples (Figure 4 and Table S3). The samples that exhibited the highest and lowest fresh weights were FZ (8:2) (12.52 g) and BFZ (8:2)–4 (2.2 g). Similarly, the highest and lowest dry weights were found in BFZ (6:4)–4 (1.88 g) and BFZ (8:2)–4 (0.3 g). The control samples exhibited fresh and dry weights of 3.53 and 0.34 g, respectively. Among the commercial nutrients, the highest fresh weight and dry weight of 8.0 and 1.1 g, respectively, were observed in nano-Zn. The lowest fresh weight and dry weight were observed in FeSO₄ (2.9 g) and Zn-EDTA (0.3 g).

Fe and Zn Contents in Groundnut Plants. The total Fe and Zn contents in the groundnut plants significantly increased due to the citrate presence, which resulted in higher mobility and higher absorption by plants through roots; the nutrient contents were even more pronounced in nanocitrates, which is more than their respective as-prepared counterparts (Table S3). More Fe was absorbed by plants than Zn during the initial days. The Fe content was higher in the BFZ (2:8)–8 samples (0.48 mg of Fe/pot), and the Zn content was higher in nano-Zn (commercial) (88.47 μg of Zn/pot) followed by ZC (1,3)–0 (82.32 μg of Zn/pot) samples. The control values were 0.02 mg of Fe/pot and 2.05 μg of Zn/pot.

DISCUSSION

Solid-state grinding followed by ball milling has been widely used for nanoparticle synthesis.^{36,37} In solid-state grinding, metal citrates were obtained from metal nitrates and citric acid alongside the evolution of nitric acid fumes. The reaction has been confirmed by nitric acid fumes, color change, and FTIR characterization. The synthesized ferric citrate was amorphous in nature as observed in XRD. The zinc citrate is crystalline in nature, but the peaks were assigned to excess citric acid added during synthesis.³⁵ Similarly, for combined metal citrates, the amorphous or crystalline nature based on the composition ratio was confirmed through the XRD technique. The FTIR, XRD, and XRF confirm the formation of metal citrates. The TEM, DLS, and sedimentation studies confirm the nanoparticle size of metal citrates and their aggregation.³⁸ The low zeta potential and high aggregation are a result of measuring in isopropanol solvent as the nanocitrates are partially soluble in water. It should also be noted that the hydrodynamic size and zeta potential will depend upon the dispersion medium, pH, and concentration.^{39,40} The difference in sedimentation rates could be due to a change in the concentration of dispersed particles. This change in the concentration may be due to rapid aggregation and disaggregation from soil extracts during sampling after incubation.⁴¹

As per the earlier reports, we observed a gradual reduction of DTPA-Fe after incubation and leaching compared to the initial days of incubation. This reduction is due to the interaction of released Fe with soil components.^{42,43} A drastic reduction in DTPA-Zn could be due to washing out during leaching or retention of Zn in soils.⁴⁴ The retention of zinc could be due to adsorption in soil particles because of fixation or aging.⁴⁵ The availability of DTPA-Fe and Zn contents varied with the ratio of Fe and Zn in citrates, ball-milling time, mobility, and so on. There was a decrease in the DTPA-Fe content with an increase in DTPA-Zn and vice versa. This observation does not depend on the composition of the combined citrates (Table S3). In combined citrates, the DTPA-Fe content decreased with the increase of the DTPA-Zn content and vice versa, which might be due to the formation of franklinite types of minerals in soil.⁴⁶ The maximum Zn⁺² solubility was furthermore related to the solubility of franklinite (ZnFe₂O₄), which was controlled by the availability of Fe³⁺ being limited by the pH-dependent solubility of Fe(III) hydroxides.⁴⁷

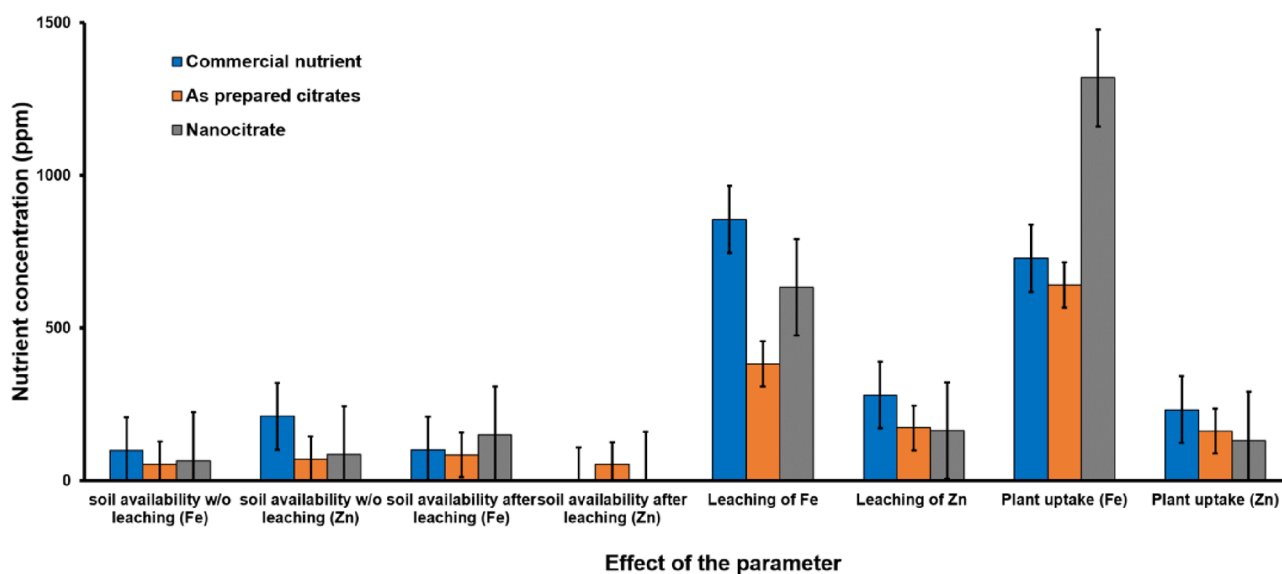


Figure 5. Comparison of studied parameters between commercial and nanocitrate fertilizers CD at 5% degrees of freedom of 11.9, and the CV is 1.53.

The type of soil, organic matter, and easy water movement through the soil provide less time for Fe or Zn sorption, resulting in a high amount of Fe and Zn leaching.⁴⁸ Sometimes, the percentage of nutrient leaching does not depend upon the nutrient application rate but on a specific soil's nutrient retention capacity. As per previous reports, any micronutrient's availability, sorption, leaching, and release depend upon soil properties.^{49,50}

Lin and Xing⁵¹ reported such inhibitory effects of nanoparticles on radish, grape, and ryegrass. The germination and vigor index improvement could be ascribed to the higher biochemical activity of plant growth enzymes due to Fe and Zn. Fe and zinc are some of the essential nutrients required for plant growth and are involved in several biochemical reactions in several crops.^{52–54} Moreover, the germination enhancement by nanocitrate samples may also be due to an increase in the water uptake capacity of the seed. It was reported earlier that it was found that nanoparticles create new pores on the seed coat, which leads to an increase in water uptake capacity.^{55,56} Even though the prepared citrates are water soluble, with the increase in the ball-milling duration, the solubility of the citrates has been reduced. The reduction in water solubility of ball-milled citrates will keep them nanosized and their entry into plants as nanoparticles.

The Zn accumulation is higher in the nano-Zn sample, which is due to its lesser leaching effect. In the ZC (1:3) sample, the high Zn accumulation in the plant is due to its high percentage of Zn content and is also less affected due to leaching. More Fe is available in the soil even after leaching. The total Fe and Zn content observations revealed that the Fe and Zn contents in shoots were less in commercial samples (FeSO₄, ZnSO₄, nano-Zn, nano-Fe, Fe-EDTA, and Zn-EDTA) compared to metal citrates, indicating that citrate NPs and their as-prepared counterparts promoted the groundnut plants. The growth promotion in the case of nanocitrates could be due to the size of the particle, mobilization, availability, and less fixation (Figure 5). Our future study will focus on the Fe and Zn contents at different phases of the plant during the crop period, which can provide more information on complete usage.

Validation. The data for all 14 characters are represented in Table S1 and were studied for 53 samples, which include additional Fe and Zn combinations (12), which were obtained directly through ferric nitrate, zinc nitrate, and citric acid at different ratios through solid-state grinding followed by ball milling.

Principal Component Analysis. The data obtained for 53 samples including different mole ratios of ferric nitrate, citric acid, and zinc nitrate were formulated and coded as FCZ (1:1:1) and FCZ (1:2:1) and were subjected to PCA. These samples were ball-milled at different durations like other ratios and subjected to PCA to unravel the underlying relationship among various plant and soil nutrient parameters. The weight ratio of Fe and Zn is almost unity and thus similar to FZ (5:5). The eigenvalue obtained from PCA for plant quality characteristics, soil nutrient, and plant nutrient content is presented (Figure 6A). The important variables in principal component analysis were the total fresh weight (g), leachate–Zn (mg/kg), leachate–Fe (mg/kg), and plant Fe content (mg/pot) for PC1, PC2, PC3, and PC4, respectively. The PC1, PC2, PC3, and PC4 contributed 31.2%, 21.8%, 10.5%, and 10.1% of the total variation, respectively (Figure 6A). The cluster analysis was performed to divide data into clusters (Figure 6B) and represent the board groups and performance. Cluster I and cluster III contain most of the samples. Cluster II contains samples that are equivalent to control treatments. Cluster I contains the best zinc, and cluster III has the best iron samples.

Ranking Index. The samples for the studied characters were ranked using the ranking index and are represented in Figure 7. The highest performer in all characters was FZ (8:2) followed by BFZ (4:6)–2. There were some negatively ranked (11 samples) among 53 samples. A negative ranking means lower performance compared to untreated and commercial checks. The studied characteristics such as the germination (%), vigor index, fresh weight, and dry weights played a major roles in the ranking index.

Cost Economics. The cost of nanocitrates and as-prepared citrates were calculated based on the concentration of raw materials used and its market price. Along with raw material

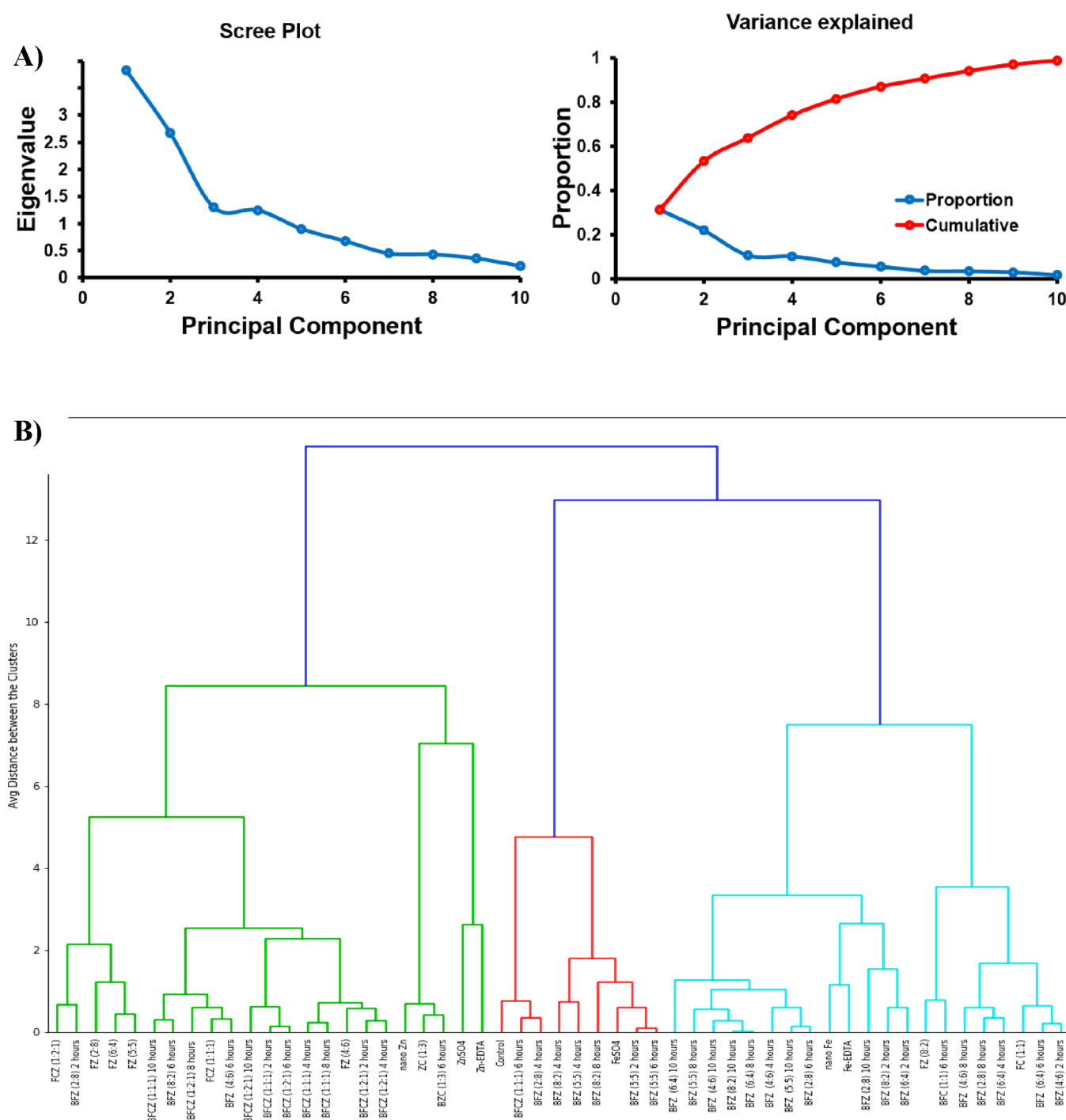


Figure 6. (A) Graphical representation of variance explained by all the principal components and (B) dendrogram depicting the relationship among 53 samples based on studied characters (scale: Euclidean distance).

prices, the cost of power consumption involved was also added as establishment charges. The cost incurred for metal citrates was USD 17.68–20.21 per kg in synthesis, and that of ball-milled citrates is USD 31.58–41.68 per kg. The cost is calculated as per the materials at our end at the smaller bench scale. The costs may vary if the ingredients have been procured at an industrial scale. The other commercial nutrients' costs were given as such procured from the market (Table S4).

CONCLUSIONS

We have successfully synthesized a series of combined citrates that is functionally valuable for higher plant use efficiency through soil application. Our results suggest that combined nanocitrates have the potential to improve seed quality and plant uptake of Fe and Zn nutrients, similar to their respective individual nutrients and other commercial fertilizers. These nanocitrates along with their as-prepared counterparts were applied on groundnut and showed maximum nutrient use efficiency and higher dry matter. It was noticed that citrate increased Fe³⁺ and Zn²⁺ nutrient uptake efficiency more than

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