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Responses of *Moringa oleifera* to alteration in soil properties induced by calcium nanoparticles (CaNPs) on mineral absorption, physiological indices and photosynthetic indicators

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Abstract

Background: The application of nanofertilisers in agriculture has been widely utilised due to their distinct characteristics and negative impacts of conventional chemical fertilisers. This study thus examined the influence of calcium nanoparticles (CaNPs) on soil composition vis-à-vis performance parameters in *Moringa oleifera* L exposed to water, 100 mg $Ca(NO_3)_2 kg^{-1}$ soil and 100, 75 and 50 mg CaNPs kg^{-1} soil. Soil morphology was determined with a scanning electron microscope coupled with energy dispersive x-ray (SEM-EDX) and elemental composition in both soils and *M. oleifera* roots determined with inductively coupled plasma-optical emission spectrometer (ICP-OES).

Results: The CaNP-amended soils were more crystalline, more fertile and had reduced salinity. An increase in immobilisation percentage of heavy metals, improvement in physiological parameters (percentage germination, vigour indices, relative water contents, lengths of roots and shoots) and photosynthetic efficiency in *M. oleifera* were recorded.

Conclusion: This study has demonstrated that CaNPs could improve soil composition for better plant performance and can act as nanofertilisers mobilising essential nutrients.

Keywords: Nanoparticles, Mineral nutrients, Photosynthetic indicators, Soil fertility, Immobilisation

1 Background

Soil is a repository of nutrients as well as a sink for pollutants. Soil chemical properties are quality indicators to determine soil fertility, soil health and exchangeable cation abilities. Soil matrix is a central medium via which vegetables absorb macro- and micronutrients required for their growth [1–3]. The efficiency of vegetable production is directly associated with the potential contribution of soil nutrients. Inadequate soil fertility requires the use of fertilisers. Ecological problems, the formation of organo-mineral precipitates and inefficient plant usage made necessary the search for alternatives to conventional agrochemicals [2, 4–9].

Applications of metal nanoparticles have remarkably improved agricultural practices as nanofertilisers to promote plant growth and enhance nutritional quality, as nanopesticides to protect against phytopathogens and as immobilising/adsorbing agents for soil pollutants [8, 10–14]. Metal nanoparticles as soil conditioners and plant growth enhancers have been reported in some studies; some had stimulatory actions while some were phytotoxic. Studies on

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stimulatory actions of metal nanoparticles showed improved antioxidant activities, increased germination percentage and longer roots and shoots whereas their phytotoxicity manifested in increased malondialdehyde level and reduced antioxidant enzyme activities [1, 9, 15, 16].

Metal nanoparticles can be used as nanofertilisers supplying essential minerals or be applied to improve the performance of conventional chemical fertilisers. They are more efficient in supplying nutrients and contribute lesser environmental pollution because of their controllable rates of release thereby reducing the risk of nutrient run-off into water [3, 5, 11–14, 17–21].

Nanoparticles in soil interact with plant roots and get translocated along with soil nutrients to other plant tissues. Their dispersal in soil facilitates the uptake of essential minerals and nutrients required for activation of enzymes such as Se and Cu for glutathione peroxidase, Mg and Fe superoxide dismutase and Fe and ascorbate [22, 23]. Nanoparticles are known to immobilise heavy metals and serve as adsorbents for pollutants [8, 12, 15]. They boost the synthesis of phytochemicals which defend plants against environmental stress and infections of pathogenic organisms. They improve chlorophyll contents, carotenoids and antioxidant activities to enrich nutritional components of vegetables [24–26].

Moreover, nanopesticidal properties of nanoparticles involve specificity towards targeted pests with little or no harmful effects on non-target or beneficial microbes, permeability to ensure availability when needed, stability to aggregation and conditional controllable release to maximise efficiency over a long period to prevent environmental pollution. These make them crop-friendly as crops would not contend with a high dose of pesticides unlike conventional pesticides [1, 8, 13, 14, 17, 19, 27–36].

Many studies have reported the effectiveness of metal and non-metal nanoparticles as nanopesticides and nanofertilisers. Nanoparticles containing Ag, Cu, Au, Mn, Fe, Ti, Zn, fullerene (C60), Mg, hydroxyapatite, Mo stimulated growth, increased biomass, elongated root and shoot length, improved vigour indices, promoted germination, reduced oxidative stress biomarkers and immobilised heavy metals in *Moringa oleifera*, *Corchorus olitorus*, *Amaranthus caudatus*, *Spinacia oleracea*, *Momordica charantia Populus deltoids*, *Solanum lycopersicum*, *Zea mays*, *Brassica juncea*, *Citrullus lanatus*, *Cucurbita pepo*, *Oryza sativa*, *Raphanus sativum*, *Arabidopsis thaliana*, *Gloriosa superba* and *Hordeum vulgare* [1, 4, 8–15, 18]. Nanoparticles containing Cu, Ag, Au, Zn, Zn/Mg, Ag/Si and Ti have also been reported to modify microbial activities in soil, suppress the population of *Aspergillus terreus*, *A. niger*, *Fusarium* spp., *Bipolaris sorokiniana*, *Phytophthora parasitica*, *Artemisia absinthium*, *Sclerotium cepivorum*, *Colletotrichum gloeosporioides*, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Trichoderma Viride*, *Alternaria alternata*, *Cladosporium* spp and exterminate *Meloidogyne* spp [21, 33, 36–40].

Moringa oleifera Linn is a source of minerals, protein, vitamins, polyphenols, fibre and carotenoids [41–45]. The presence of soil pollutants can hinder bioavailability of essential nutrients needed by *M. oleifera* for growth.

Calcium nanoparticles (CaNPs) can assist in improving soil fertility, assist in the mobility of some trace mineral elements and assist in the probable immobility of heavy metals which are of no biological interest [36, 37, 46]. $Ca_3(PO_4)_2$ nanoparticles have been reported to enhance root and shoot elongations, improve antioxidant enzyme activities and reduce lipid peroxidation levels in rice [40].

Evidence on functions of zero-charged calcium nanoparticles (CaNPs) mediated with $Ca(NO_3)_2$ and pod extract of Kola (*Cola nitida* S) on soil composition, heavy metal remediation, mobilisation of essential minerals, improvement of plant physiology and as photosynthetic indicators has yet to be reported since nanoparticles undergo different transformations in soil. The study was undertaken to determine the influence of CaNPs on soil properties, nutrient/fertility status and stimulatory/phytotoxicity potentials on *M. oleifera* planted on amended soils with CaNPs as an alternative to conventional fertilisers.

2 Methods

2.1 Green synthesis of CaNPs and determination of its point of zero charge

CaNPs were synthesised using the pod extract of *Cola nitida*, which was obtained by extracting 1.0 g of dried pod in 100 ml of water at 60 °C for 1 h. Thereafter, 15 ml of the extract was reacted with 150 ml of 1 mM $Ca(NO_3)_2$ at 60 °C for 1 h, leading to the formation of a deep golden brown colloidal solution, which absorbed maximally at 215 nm. Synthesised CaNPs were characterised using transmission electron microscopy, Fourier Infra-red spectroscopy and UV-Visible spectroscopy.

The pH point of zero charge (pHpzc) of 100, 75 and 50 mgL^{-1} CaNPs and 100 mgL^{-1} $Ca(NO_3)_2$ was determined by adding 10 ml of each concentration separately to 200 ml 0.1 M NaCl with known pH. The pH was adjusted between 1 and 10 with 0.1 M NaOH or 0.1 M HCl. Final pH values were taken after 24 h with Jenway 6405 pH meter (Germany). A plot of the difference in pH (final – initial) against initial pH was made and the point of intersection on the horizontal axis is the pHpzc.

2.2 Soil and plant collection, analysis and morphological characteristics

Soil samples were collected from a farm located on latitude 7° 759502′ N and longitude 4° 599194′ E at a depth between 0 and 25 cm, air dried, pulverised and sieved with a 600-μm wire mesh.

Methods described by [47, 48] were used for the analysis of soil pH, organic carbon, phosphorus, nitrogen, soil texture and composition and cation exchange capacity. Exchangeable sodium percentage (ESP) was calculated with Eq. 1. ESP is a useful measure of Na on soil properties.

$$ESP = {Na}\Big/{[K + Ca + Mg + Na]} \quad (1)$$

Mineral and heavy metal analysis was done by digesting 1 g of air-dried pre-planting, post-harvesting soil and *M. oleifera* root separately with a mixture of concentrated HNO_3 and HCl (7:3). The solution was boiled at 100 °C, allowed to cool, filtered and made up to 20 ml with deionised distilled water. The soil sample was analysed in triplicate.

An inductively coupled plasma with optical emission spectrometer (ICP-OES, Agilent 720-ES, USA) was employed for the analysis of Na, K, Ca, Mg, Zn, Cu, Fe, Ni, Mn, Pb, Cd, Cr and As in soil. The instrument was rinsed with 5% HNO_3 after which blank was run and then targeted metals without interference at emission lines Zn (213.857), Cu (327.395), Pb (220.353), Ni (231.604), Fe (238.204), Cd (214.439), Mn (217.610), Cr (267.716), Mg (279.553), Ca (396.847), Na (589.592), K (766.491) and As (188.980) were analysed. Reproducibility of the instrument was ensured by plotting calibration curves of metal standards with regression equation R^2 = 0.995.

Morphological characteristics and elemental composition of both pre-planting and post-harvesting soil were determined using scanning electron microscopy and energy dispersive x-ray (SEM-EDX, JEOL JSM-7600F).

Adsorption (immobilisation) capacity of amended soils for heavy metal was calculated with Eq. 2.

$$Heavy\ metal\ adsorption = \frac{Concentration\ in\ pre-planting\ soil - concentration\ in\ post-harvest\ soil}{Concentration\ in\ pre-planting\ soil}\ x\ 100 \quad (2)$$

2.3 CaNPs amendment of soil and planting of *M. oleifera* seeds

Twenty-five (5 for each group) non-perforated buckets (75 mL) were filled each with 250 g of air-dried, pulverised and filtered soil. *Moringa oleifera* seeds were exposed to water, 100 mg $Ca(NO_3)_2$ kg^{-1} soil, 100 mg CaNPs kg^{-1} soil, 75 mg CaNPs kg^{-1} soil and 50 mg CaNPs kg^{-1} soil as groups A, B, C, D and E respectively for 3 weeks. Groups A and B served as positive and negative controls respectively. *M. oleifera* plants were maintained under day and night cycles of 12 h for 3 weeks at a UV index (5.11 ± 1.15), temperature (29.14 ± 0.06 °C), relative humidity (35.87 ± 2.14%) and light intensity (13255 ± 40.92 illuminance).

2.4 Determination of germination indices in *M. oleifera*

M. oleifera were planted to maturity and harvested after 3 weeks. Percentage germination, root and shoot lengths, vigour index and relative water contents were determined in *M. oleifera* as previously reported by [8, 12].

2.5 Mineral nutrients in roots and absorption percentage in *M. oleifera* plant

Di-acid digestion using HNO_3:$HClO_4$ (9:4) was used for digesting 0.5 g each of dried (70 °C for 2 h) *M. oleifera* root in 10 ml di-acid. The solution was placed on a heating mantle programmed at 85 °C, increased to 120 °C and heated continuously until the fumes ceased. It was made up to 20 ml with deionised-distilled water. Mineral and heavy metal contents were determined using ICP-OES as described in Section 2.2.

Metal absorption from the soil by root was calculated using Eq. 3 [8].

$$Percentage\ metal\ absorption = \frac{metal\ content\ in\ root}{metal\ content\ in\ soil}\ x\ 100 \quad (3)$$

2.6 Photosynthetic pigment contents in *M. oleifera*

Chlorophyll a, b and carotenoid contents were measured in *M. oleifera* as described by [49]. 0.1 g of the fresh leaves was homogenised in 5 ml ice-cold 80 % acetone, centrifuged at 5000 rpm for 5 min and then re-extracted with 2.5 ml of ice-cold 80 % acetone twice. The absorbance of the combined extract was measured at 470, 663, 645 nm and their quantities expressed as mg/g FW calculated using Eqs. 4–6.

$$Chlorophyll\ a = 12.25\ x\ A_{663} - 2.79\ x\ A_{645} \quad (4)$$

$$Chlorophyll\ b = 21.50\ x\ A_{645} - 5.10\ x\ A_{663} \quad (5)$$

(Total chlorophyll = Chlorophyll a + chlorophyll b)

$$Carotenoid = \frac{(1000\ x\ A_{470} - 1.82\ x\ Chl\ a - 85.02\ x\ Chl\ b)}{198} \quad (6)$$

2.7 Statistical analysis

Results of mineral contents, photosynthetic pigments and relative water contents are expressed as mean ± standard deviation of three replicates. Results of root length, shoot length, vigour index, number of leaves and percentage germination are expressed as mean ±

standard deviation of fourteen replicates. These results were subjected to one-way ANOVA. Duncan's multiple range test was used for the comparison of means. The level of significance was performed at $p < 0.05$ using IBM SPSS 20 version.

3 Results

3.1 Biosynthesis of CaNPs

Calcium nanoparticles (CaNPs) mediated using the pod extract of *C. nitida* are a deep golden brown colloidal solution which absorbed maximally at 215 nm. It is made up of a meshwork of particles of about 80 nm in size (Fig. 1a). FTIR spectrum of CaNPs showed peaks at 3424 cm^{-1} indicating N-H vibrations and at 1711 cm^{-1} suggesting C=O of ketone (Fig. 1b). This implies the presence of biomolecules needed for capping and stabilising nanoparticles in the extract of *C. nitida*. The colloidal CaNPs were used as obtained from the Laboratory of Industrial Microbiology and Nanobiotechnology, LAUTECH, Ogbomoso, Nigeria, except for dilution to obtain necessary concentrations for the study.

pH point of zero charge (pH_{pzc}) of $Ca(NO_3)_2$, 100, 75 and 50 mgL^{-1} CaNPs are 6.58, 6.51, 6.51 and 6.50 respectively. At $pH < pH_{pzc}$, the surfaces of these solutions will be cationic and at $pH > pH_{pzc}$, their surfaces will be anionic.

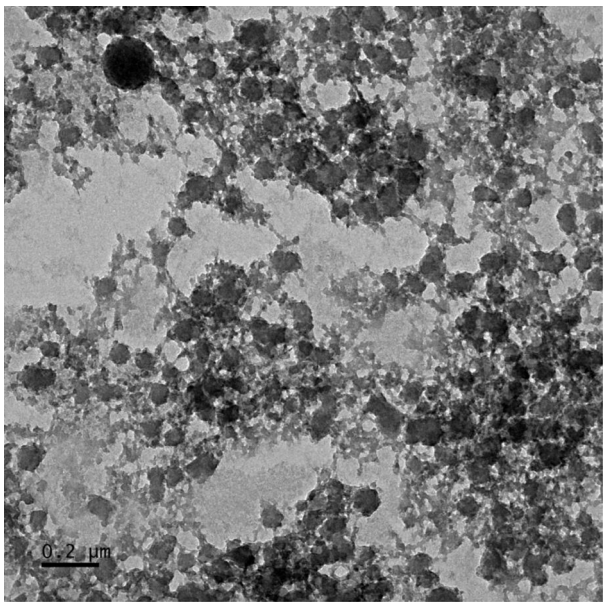
3.2 Soil quality indicators, elemental composition, morphology and adsorption

Soil quality indicators are essential parameters to measure fertility and their consequences on plant growth in addition to contributions to other activities involved in plant germinations and sustenance. Pre-planting (raw) soil in this study (Table 1) was inherently dark, sandy loam, slightly silty, slightly acidic with a sufficiently good percentage of organic carbon and organic matter soil texture. CaNPs beneficially enhanced nitrogen contents by 6.73, 5.18 and 2.28 % in C, D and E respectively (Table 1). Nitrogen contents were significantly higher in soils amended with $Ca(NO_3)_2$ and all concentrations of CaNPs while A (water) had comparable N contents with F (pre-amended) (Table 1). Phosphorus contents in all soils were comparable with soil C amended with CaNPs having a slightly higher content (1.9 %) than F while others had minimally reduced contents. Statistically significant ($p < 0.05$) decrease in organic matter and percentage organic carbon in groups A, B, D and E were obtained compared to F although C had a comparable organic matter and percentage organic carbon contents to pre-amended (F) but with a slight decrease (Table 1). An insignificant ($p > 0.05$) increase in pH was obtained for soil amended with 100 mg $Ca(NO_3)_2$, 100 (group C), 75 (group D) and 50 mgL^{-1} CaNPs (group E) whereas a slight decrease was recorded for soil watered with water compared to pre-amended soil (F). Increase in soil pH was not concentration-dependent. The pH values of respective soils amended with these solutions are higher than respective pH_{pzc} of CaNPs and $Ca(NO_3)_2$ indicating their surface charges in the soil possibly as anionic. Percentage of clay, silt and sand in amended soil remained largely similar to pre-amended soil.

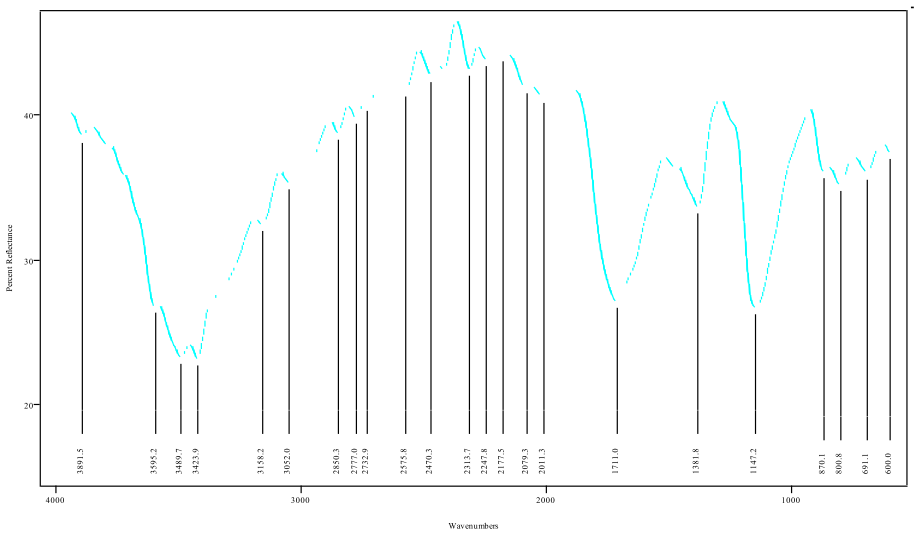
Raw soil had reasonably moderate available N, P, Cu, Mn, Zn, Fe, K, Ca, Mg, Na and Be as well as a poor source of Cd, Cr, Pb and As. Abundance of mineral elements in raw soil follows K > Fe > Ca > Mg > Na > Cu > Zn > Mn > Cd > Pb > Cr > Be > Cr > As. Concentrations of K, Zn, Na, Cu, As, Pb, Cd, Cr and Ni (Table 1) were significantly ($p < 0.05$) reduced in amended soils in comparison with F (raw). Concentrations of Ca were significantly ($p < 0.05$) higher in soils B, C, D and E than in F while A had comparable concentration with F. Concentrations of Fe, Mn and Mg were statistically insignificantly ($p > 0.05$) altered in all soils except for Mn in soil E compared with F (Table 1). Availability of macro and micronutrients such as Ca, N and Fe in amended soil were higher than in pre-amended whereas the presence of toxic heavy metals was consequently immobilised.

Raw soil cation exchange capacity (CEC) was determined as 8.06 ± 0.66 $cmol_cKg^{-1}$ with a calculated exchangeable sodium percentage (ESP) of 0.11 $cmol_cKg^{-1}$. Soils in B, D and E had a comparable proportion of CEC as F. Group C had significantly ($p < 0.05$) greater CEC than F while A had significantly lower CEC than F. CaNPs beneficially improved CEC by 2.81 % and 11.27 % over F and A respectively. Higher CEC in soil amended with 100 mgL^{-1} CaNPs is an indication of a highly reactive and more negatively charged the soil.

Raw soil had a semi-porous nearly closely packed oval morphological structure (Fig. 2) with presence of O (29.45 %), Si (34.80 %), Ca (5.21%), Na (7.90 %), Mg (2.40 %), N (3.66 %), C (9.84 %) and Al (0.84 %) as obtained in EDX (Table 2). Conspicuous alterations in morphological characteristics (Fig. 2) were recorded in SEM images of amended soils. A more crystalline soil with hollows was recorded for A and D even though, A had more pores than D which appeared more fused. A sheet-like with well carved-out cavities was obtained for B; a sponge tissue-like soil structure with most-developed pores for C and a rod-like organised structure was recorded for E. Energy dispersive X-ray analysis (Fig. 2, Table 2) revealed an improved percentage O in A, B, D and E with a slight reduction in C compared with raw. All groups had decreased Si with C having comparable content with respect to F. Soil C had higher Ca, lower Na than other soils and more importantly the presence of Ag mobilised from the soil matrix. Results of elemental compositions (Table 2) determined with EDX show similarities with ICP-OES data.



a



b

Fig. 1 **a** The TEM micrograph of biosynthesised CaNPs. **b** FTIR spectrum of calcium nanoparticles mediated with *Cola nitida*

A measure of Na influence (Table 1) on soil properties was significantly ($p < 0.05$) restricted in C and E. A significant decrease in exchangeable sodium percentage (ESP) and increase in Ca in the soil are an indication pointing to lower salinity of amended soils.

3.3 Influence of CaNPs on *M. oleifera* germination parameters

Germination indicators are quality metrics to gauge the importance of different concentrations of CaNPs and ($Ca(NO_3)_2$) as compared with water (control) on modulation on tolerance ability of *M. oleifera*. Noteworthy variations with better physiological expressions in germination percentages, number of leaves, root and shoot lengths (Fig. 3) in *M. oleifera* planted on zero-charged CaNP-amended soil (C, D and E) in comparison with water (A) and $Ca(NO_3)_2$ (B) were observed.

Amendment with 75 and 100 mgL^{-1} CaNPs significantly ($p < 0.05$) increased shoot and root lengths with groups B ($Ca(NO_3)_2$) and E (50 mgL^{-1} CaNPs) having comparable root and shoot lengths to A (control) (Table 3). The 75 mgL^{-1} CaNPs had 28.98 and 16.67 % improvements in shoot and root lengths respectively while the 100 mgL^{-1} CaNPs had enhanced shoot and root lengths with 42.69

Table 1 Analysis of pre-planting and post-harvest soils with ICP-OES

Soil characteristics	A	B	C	D	E	F
pH (H_2O)	6.53 ± 0.03^{a}	6.67 ± 0.07^{a}	6.62 ± 0.02^{a}	6.62 ± 0.04^{a}	6.62 ± 0.12^{a}	6.59 ± 0.03^{a}
Organic carbon (%)	454.28 ± 14.27^{a}	442.50 ± 22.16^{b}	463.07 ± 16.97^{c}	453.86 ± 42.06^{a}	$447.14 \pm 39.66^{a,b}$	464.31 ± 21.21^{c}
Organic matter (%)	783.18 ± 24.60^{a}	762.83 ± 30.23^{b}	798.98 ± 29.26^{c}	782.17 ± 72.51^{a}	770.89 ± 68.37^{d}	800.47 ± 36.57^{c}
Clay (%)	12.22 ± 1.05^{a}	12.63 ± 0.86^{a}	12.84 ± 1.42^{a}	12.81 ± 0.56^{a}	12.26 ± 0.79^{a}	12.85 ± 1.37^{a}
Silt (%)	29.19 ± 0.89^{a}	29.29 ± 0.04^{a}	29.36 ± 1.44^{a}	29.40 ± 0.84^{a}	29.23 ± 0.66^{a}	29.51 ± 1.08^{a}
Sand (%)	58.59 ± 0.95^{a}	58.07 ± 1.17^{a}	58.34 ± 0.99^{a}	57.79 ± 0.27^{a}	58.66 ± 1.12^{a}	57.64 ± 0.86^{a}
CEC ($cmol_cKg^{-1}$)	7.54 ± 0.22^{a}	7.98 ± 0.82^{b}	8.39 ± 0.76^{c}	7.93 ± 0.61^{b}	8.01 ± 0.67^{ab}	8.16 ± 0.66^{b}
N ($mgkg^{-1}$)	544.18 ± 7.14^{a}	573.33 ± 6.99^{b}	576.68 ± 5.44^{b}	$568.32 \pm 4.06^{b,c}$	552.43 ± 7.87^{c}	540.31 ± 6.32^{a}
P ($mgkg^{-1}$)	13.45 ± 0.55^{a}	12.95 ± 0.15^{a}	13.94 ± 0.88^{a}	13.13 ± 0.63^{a}	12.90 ± 0.01^{a}	13.68 ± 0.18^{a}
K ($mgkg^{-1}$)	91.20 ± 0.03^{a}	92.44 ± 0.02^{a}	93.07 ± 0.07^{a}	98.50 ± 0.05^{b}	96.68 ± 0.72^{b}	108.24 ± 0.08^{c}
Na ($mgkg^{-1}$)	16.21 ± 0.05^{a}	$18.97 \pm 1.07^{a,b}$	14.16 ± 0.17^{c}	$17.57 \pm 0.07^{a,b}$	15.33 ± 0.09^{a}	19.31 ± 0.17^{b}
Ca ($mgkg^{-1}$)	38.09 ± 0.01^{a}	43.40 ± 0.03^{b}	44.18 ± 0.00^{b}	$40.43 \pm 0.00^{a,b}$	$41.17 \pm 0.02^{a,b}$	38.38 ± 0.00^{a}
Mg ($mgkg^{-1}$)	29.86 ± 0.77^{a}	31.09 ± 0.59^{a}	$32.88 \pm 0.18^{a,b}$	34.21 ± 0.22^{b}	31.19 ± 0.02^{a}	34.23 ± 0.07^{b}
Be ($mgkg^{-1}$)	nd	0.0029 ± 0.00^{a}	0.0008 ± 0.00^{b}	0.0021 ± 0.00^{a}	0.0005 ± 0.00^{b}	0.0658 ± 0.00^{c}
Fe ($mgkg^{-1}$)	51.80 ± 0.20^{a}	50.12 ± 0.06^{a}	52.45 ± 0.34^{a}	52.79 ± 0.59^{a}	51.12 ± 0.24^{a}	51.00 ± 0.74^{a}
Mn ($mgkg^{-1}$)	1.65 ± 0.01^{a}	1.62 ± 0.00^{a}	1.63 ± 0.01^{a}	1.29 ± 0.02^{b}	1.13 ± 0.01^{c}	1.67 ± 0.01^{a}
Zn ($mgkg^{-1}$)	1.30 ± 0.00^{a}	1.45 ± 0.00^{b}	$1.38 \pm 0.00^{a,b}$	1.58 ± 0.00^{c}	$1.37 \pm 0.00^{a,b}$	1.77 ± 0.01^{d}
Cu ($mgkg^{-1}$)	1.23 ± 0.02^{a}	1.47 ± 0.00^{b}	1.43 ± 0.01^{b}	1.93 ± 0.37^{c}	1.51 ± 0.01^{b}	1.94 ± 0.54^{c}
Ni ($mgkg^{-1}$)	0.038 ± 0.00^{a}	0.035 ± 0.00^{a}	0.017 ± 0.00^{b}	$0.041 \pm 0.01^{a,c}$	0.024 ± 0.00^{b}	0.049 ± 0.01^{c}
Cd ($mgkg^{-1}$)	0.49 ± 0.00^{a}	0.108 ± 0.00^{b}	0.107 ± 0.00^{b}	0.108 ± 0.00^{b}	0.102 ± 0.00^{b}	0.69 ± 0.00^{c}
Pb ($mgkg^{-1}$)	0.25 ± 0.01^{a}	$0.21 \pm 0.01^{a,b}$	0.17 ± 0.00^{b}	0.18 ± 0.00^{b}	0.19 ± 0.01^{b}	0.32 ± 0.01^{c}
Cr ($mgkg^{-1}$)	0.18 ± 0.00^{a}	0.15 ± 0.01^{a}	0.14 ± 0.00^{a}	0.143 ± 0.01^{b}	0.127 ± 0.01^{b}	0.193 ± 0.01^{c}
As ($mgkg^{-1}$)	nd	0.108 ± 0.00^{a}	nd	nd	0.0030 ± 0.00^{b}	0.184 ± 0.01^{c}
ESP ($cmol_cKg^{-1}$)	$0.095 \pm 0.01^{a,c}$	0.103 ± 0.01^{a}	0.078 ± 0.00^{b}	$0.094 \pm 0.00^{a,c}$	0.087 ± 0.00^{c}	0.100 ± 0.00^{a}

Group A: water (control), group B: 100 mg $Ca(NO_3)_2$/kg soil, group C: 100 mg CaNPs/kg soil, group D: 75 mg CaNPs/kg soil, group E: 50 mg CaNPs/kg soil, F: pre-planting (raw) soil. Results are expressed as mean ± standard deviation of three replicates. Results having different superscripts across the rows are significantly different ($p < 0.05$). *nd*, not detected

and 35.09 % respectively (Table 3). Percentage germination improved significantly ($p < 0.05$) in C and D by 40.18 and 29.28 % while marginal non-significant ($p > 0.05$) modulation was observed in B (8.91 %) and in E (4.31 %). Leaves (number) flourished significantly ($p < 0.05$) better by 40.99 % in C and 13.95 % in D whereas B had a statistically comparable number of leaves to A except for E that had a slight decrease. Relative water contents were significantly ($p < 0.05$) higher in C and D than A while E and B had comparable contents to A (Table 3). Vigour indices of groups B, C and D were significantly higher than A but E had a lower index than A.

3.4 Stimulatory influence of zero-charged CaNPs on carotenoid and total chlorophyll contents

Total chlorophyll contents of *M. oleifera* planted on CaNPs were significantly improved (Table 3) compared to A. Group C had the highest chlorophyll a followed by group D and E. The main photosynthetic pigment (chlorophyll a) was CaNP concentration-dependent. Similarly, $Ca(NO_3)_2$ (group B) had significantly higher chlorophyll b than group A but chlorophyll a content was comparable even though lower by 14.38 %. Chlorophyll b contents follow groups C > B > D > E > A.

The indicator of efficient photosynthetic activity (Fig. 4) was inverse-CaNP concentration-dependent. $Ca(NO_3)_2$ (B) was least efficient while 50 mgL^{-1} was most efficient in boosting photosynthetic activities in *M. oleifera*.

Concentration-dependent stimulatory influences of CaNPs on carotenoid contents in *M. oleifera* (Table 3) are significantly ($p < 0.05$) pronounced in groups C, D and E with group B having insignificantly ($p > 0.05$) lower contents compared to group A.

3.5 Impact of CaNPs on *M. oleifera* root mineral nutrient, heavy metal absorption and adsorption

For macronutrients in roots (Table 4), concentrations of Ca and Mg were statistically significantly ($p < 0.05$) improved in all CaNP-amended soils along with K in

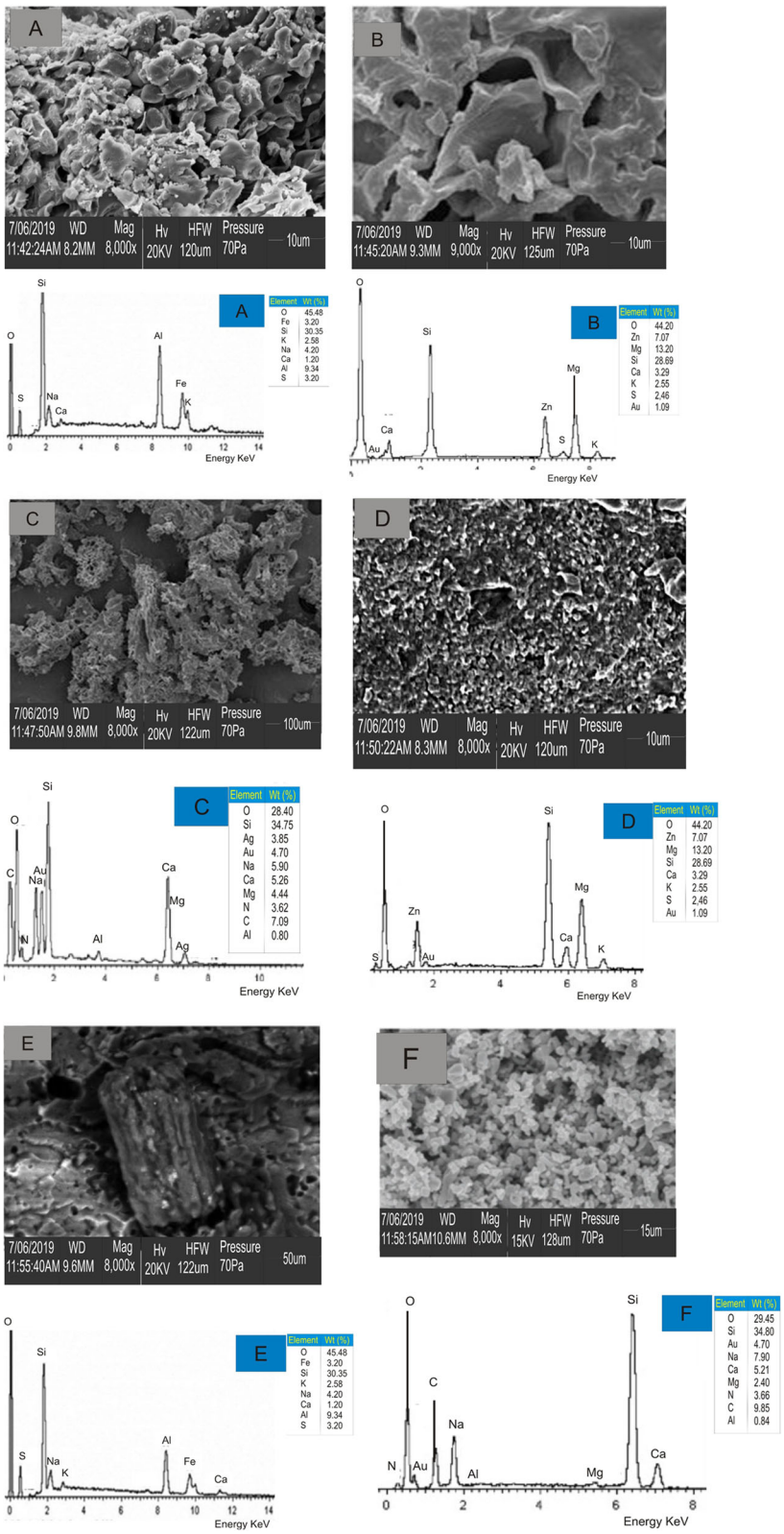


Fig. 2 SEM and EDX images of raw and amended soil samples. Group: A water (control), group B: 100 mg Ca(NO3)2/kg soil, group C: 100 mg CaNPs/kg soil, group D: 75 mg CaNPs/kg soil, group E: 50 mg CaNPs/kg soil, F- pre-planting (raw) soil

Table 2 Elemental composition (Weight %) of pre-planting and post-harvest soils using EDX

Elements	A	B	C	D	E	F
O	45.48	44.20	28.40	44.20	45.48	29.45
Si	30.35	28.69	34.70	28.69	30.35	34.80
Fe	3.20	–	–	–	3.20	–
K	2.58	2.55	–	2.55	2.58	–
Na	4.20	–	5.90	–	4.20	7.90
Ca	1.20	3.29	5.26	3.29	1.20	5.21
Al	9.34	–	0.8	–	9.34	0.84
S	3.20	2.46	–	2.46	3.20	–
N	–	–	3.62	–	–	3.66
Zn	–	7.07	–	7.07	–	–
Mg	–	13.20	4.44	13.20	–	2.40
Ag	–	–	3.85	–	–	–
C	–	–	7.09	–	–	9.85
Au	–	–	4.70	1.09	4.70	–

Group A: water (control), group B: 100 mg $Ca(NO_3)_2$/kg soil, group C: 100 mg CaNPs/kg soil, group D: 75 mg CaNPs/kg soil, group E: 50 mg CaNPs/kg soil, F: pre-planting (raw) soil

groups D and E. Absorption of Na was significantly ($p < 0.05$) suppressed in C, D and E to the tune of 31.39, 15.26 and 19.94 % respectively while an insignificant minimal reduction was obtained for B.

Root absorption of macronutrients by *M. oleifera* from soil (Table 4) increased K by 1.23, 3.03, 0.48 and 5.16 % together with Ca by 0.12, 1.53, 5.30 and 0.4 % in B, C, D and E respectively over control (A). Percentage of Mg absorbed in root only increased in C and E; others had decreased contents compared to A. Percentage of absorption of Na declined by 15.82, 21.23, 21.56 and 15.24 % in B, C, D and E in contrast to A (control).

Micronutrient concentrations (Table 4) follow the trend Fe > Zn > Cu > Mn in *M. oleifera* planted on amended and control soil. Levels of Fe, Mn and Zn absorbed by roots of *M. oleifera* increased significantly ($p < 0.05$) in B and C alongside Fe in E and Cu in C. Other groups had nearly similar concentrations compared to A (control). Percentage of Mn absorption in roots of *M. oleifera* improved by 12.93, 13.97, 24.92 % in B, C and E respectively followed by Fe by 3.84, 9.02, 6.47, 9.44 % in B, C, D and E respectively then in Zn by 4.95 and 7.74 % in B and C respectively in addition to Cu by 2.27 % in C over A.

Availability of heavy metals found in the roots of *M. oleifera* (Table 4) ranged as follows: Pb > Cd > Cr > Ni. Significant ($p < 0.05$) reductions in contents of Ni in C, Pb in B and C and Cd in B, C, D and E together with Cr in E were obtained. Arsenic (As) was not detected in all roots except in B. Percentage of Cd absorbed by *M. oleifera* roots (Table 4) decreased by 4.3, 16.28, 13.88 and 16.1 % in B, C, D and E respectively and Pb by 4.02, 13.76 and 7.49 % in B, C and D respectively. Increased root absorption of Ni was found in all groups except for D. Likewise, Cr absorption increased in B and D although, a minimal reduction was found for C and E. Except for B, As was not absorbed by roots.

Phytoremediation (immobilisation) percentages (Table 4) in A are 6.87 19.49, 21.71, 28.82, and 100 for Cr, Ni, Pb, Cd, and As respectively. Immobilisation percentages improved to 28.45, 84.36, 36.13, 21.57 % for Ni, Cd, Pb, Cr although with reduced immobilisation of As by $Ca(NO_3)_2$ as compared to A. CaNPs further enhanced percentages immobilisation to 64.71, 84.51, 47.24, 28.83 and 100 in C; 17.14, 84.38, 46.03, 26.03 and 44.02 in D and 52.40, 85.31, 40.40, 34.07 and 98.37 % in E for Ni, Cd, Pb, Cr and As respectively.

4 Discussion

Vegetable production efficiency is directly associated with the potential contribution of soil nutrients. Insufficient harmonisation of soil nutrients occasioned by different agricultural practices has created a vacuum for the use of agrochemicals. Agrochemicals undoubtedly assist to replenish lost nutrients; however; ecological drawbacks and precipitation of ineffectual organo-minerals are some of their shortcomings [2, 4–9, 12, 15]. Moreover, metal nanoparticles as alternatives to agrochemicals acting as soil conditioners and plant growth enhancers have been reported in several studies [1, 9, 16].

Our results indicate that soils amended with CaNPs were structurally stable having similar percentages of clay, silt and sand although were more porous and in some cases more crystalline that could aid translocation as well as absorption of nutrients. CaNPs altered significantly the morphologies of amended soil to increase porosity, improve soil surface reactivity, enhance water absorption, nutrient and CaNP translocation. Similar observations were recorded by [50, 51]. Porosity is an influential factor that determines soil viability and productivity and has a direct association with water holding capacity (WHC). Water holding capacity of soil is one of the parameters of importance in plant growth sustenance [2, 51]. Originally, raw soil in this study was composed of low porous compact soil aggregate that changed significantly with the introduction of CaNPs. Nanoparticles are known to interact with soil by altering agglomeration and aggregation rates to facilitate soil/nanoparticles granules into easily transportable units through modification of surface charges [7, 46].

Soil chemical properties are quality indicators to determine the fertility, health and exchangeable cation abilities of soil [2]. Amended soils are slightly less acidic

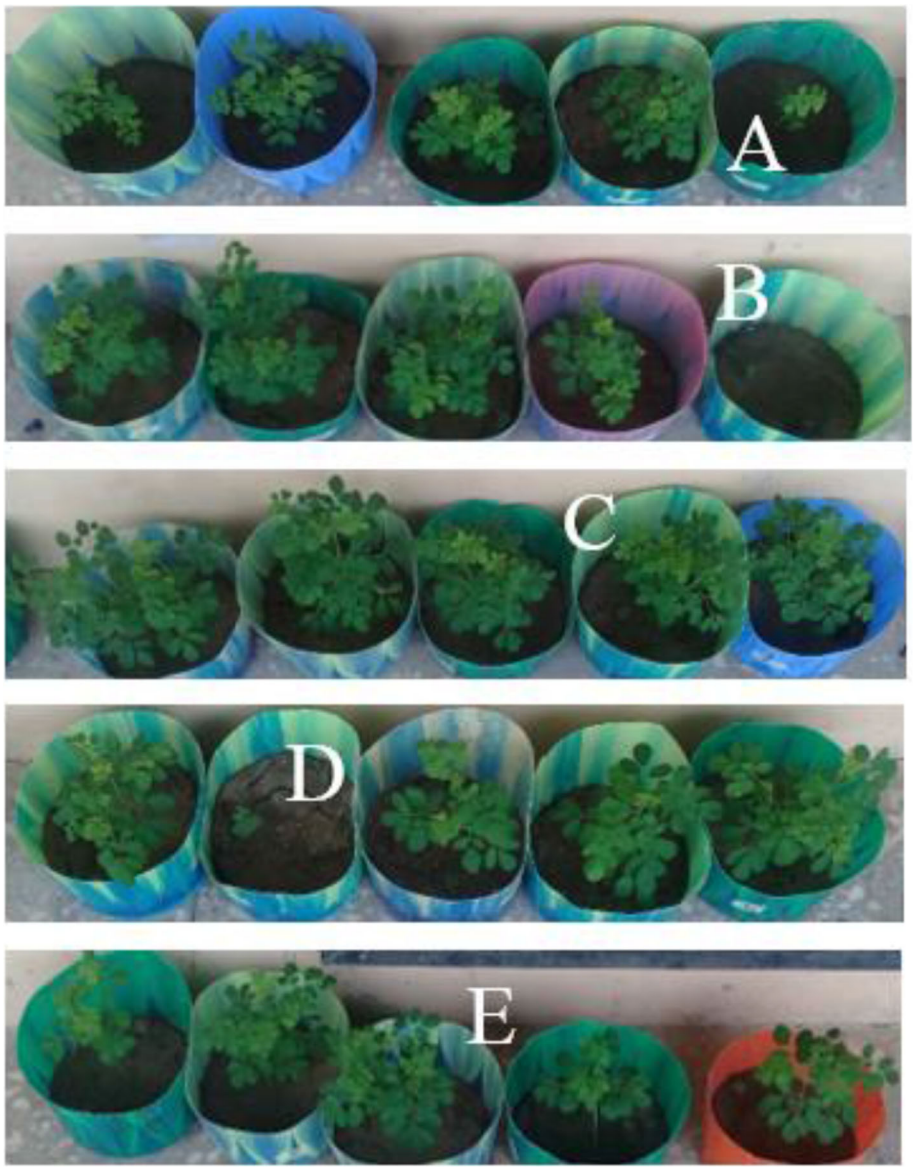


Fig. 3 Growth patterns of *M. oleifera* on raw and amended soil samples. Group A: water (control), group B: 100 mg Ca(NO3)2/kg soil, group C: 100 mg CaNPs/kg soil, group D: 75 mg CaNPs/kg soil, group E: 50 mg CaNPs/kg soil, F- pre-planting (raw) soil

that might have resulted from amendment since the pH of CaNPs were higher. pH is a determining factor that affects soil nutrient availability, microbial processes and mobility of toxic trace elements [23, 52, 53]. Useful macronutrients such K, Ca, N, P and some micronutrients like S, Cu, Mn, Fe are usually more mobile between pH 6.5–7.5 especially with an increase in pH towards 7.5 whereas toxic trace metals mobility would be more retarded [7, 46]. Nanoparticles have abilities to alter soil pH by changing concentrations of H^+ or OH^- in soil pore water [26, 50, 54]. This implies that the slight increase in pH could have contributed to additionally available macronutrients obtained in this study and perhaps higher immobilisation of heavy metals.

Amended soils are as rich in organic matter and organic carbon as raw soil particularly amended soil with 100 mgL^{-1} CaNPs though with higher CEC. Organic matter is the reservoir of soil fertility and consequentially influential in sustaining the soil ecosystem [7, 55]. The largely unchanged compositions of organic matter by CaNPs amendment is connected to the lengthy period it would take for complete mineralisation of soil for a noticeable change in soil organic contents. This is in consonance with results of [26, 46, 54, 56] that

Table 3 Germination and physiological indices of *M. oleifera* grown under different soil conditions

Groups	Root length	Shoot length	number of leaves	Vigour index	Percentage germination	Relative water content	Photosynthetic pigments (mg/g of fresh weight)		
							Chlorophyll a	Chlorophyll b	Carotenoids
A	3.42 ± 0.16 [a]	10.94 ± 1.14 [a]	41.86 ± 2.51 [a]	826.28 ± 7.18[a]	57.54 ± 5.52 [a]	36.77 ± 0.65 [a,d]	1.53 ± 0.33 [a]	1.35 ± 0.92 [a]	3.22 ± 0.68 [a]
B	3.38 ± 0.06 [a]	12.46 ± 0.08[b,c]	45.57 ± 1.48 [a,c]	986.67 ± 3.36[b]	62.29 ± 4.17[a]	31.03 ± 0.93[a]	1.31 ± 0.44 [a]	2.41 ±0.78 [b]	3.03± 0.23 [a]
C	4.62 ± 0.26 [b]	15.61 ± 1.51[c]	59.02 ± 3.12 [b]	1631.75 ± 11.81[c]	80.66 ± 2.88[c]	63.60 ± 04.72[b]	4.45 ± 0.35 [c]	3.66 ± 0.03 [c]	6.07 ± 0.47 [b]
D	3.99 ± 0.43[c]	14.11 ± 1.21[c]	47.71 ± 0.82 [c]	1319.68 ± 2.15 [d]	74.39 ± 1.33[d]	58.49 ± 6.32[c]	3.24 ± 0.09 [b]	2.20 ± 0.19 [b]	4.98 ± 0.32 [c]
E	3.63 ± 0.39[a]	10.66 ± 0.03 [a]	40.43 ± 2.89 [a]	819.27 ± 1.75[a]	60.02 ± 4.28[a]	39.03 ± 3.33[d]	2.42 ± 0.22 [c]	1.59 ± 0.25 [a]	4.18 ± 0.82 [d]

Group A: water (control), group B: 100 mg $Ca(NO_3)_2$/kg soil, group C: 100 mg CaNPs/kg soil, group D: 75 mg CaNPs/kg soil, group E: 50 mg CaNPs/kg soil. Results of percentage germination, root length, shoot length, relative water contents, vigour index and growth tolerance index are expressed as mean ± standard deviation of fourteen replicates while number of leaves was expressed as mean ± standard deviation of leaves on fourteen replicates. Results having different superscripts along the column are significantly different ($p < 0.05$)

reported unchanged composition of organic matter following the addition of CuO and Fe_3O_4 nanoparticles but contrary results were reported by [51]. Nanoparticles are known to interpose within soil organic matter and clay to form aggregates that can easily be transported. Nanoparticle mobility is predicted by dispersibility and electrostatic attraction/repulsion between soil and charges on nanoparticles. There would be repulsion between negative soil surface charges and negative surfaces of CaNPs (pH_{pzc} value), thus increasing the mobility of CaNPs into plants. Hence, the closer the difference between soil pH and pH_{pzc} of nanoparticles, the more mobile the nanoparticles are into plant roots. Also, the more porous the soil is, the less retained the nanoparticles are [50, 56]. This is in agreement with the results of soil porosity as obtained in this study indicating CaNPs were most mobile in soil amended with 100 mgL^{-1} CaNPs. This agrees with reports of [24, 50, 51]. Furthermore, higher CEC in soil amended with 100 mgL^{-1} CaNPs suggests a highly reactive and more negatively charged soil [1, 2, 51, 57]. CEC provides soil fertility status vis-à-vis its nutrient retention. As obtained in this study, it is clear that 100 mgL^{-1} CaNPs improved soil ability to adsorb more nutrients and by extension an indication of soil quality and productivity [2, 46, 51].

Amended soils are beneficially richer in nitrogen than raw. The improvement in N content might be credited to the release of nitrogen from CaNPs (mediated by the pod extract of *C. nitida* using $Ca(NO_3)_2$ salt) and nutrient mobilisation by the nanoparticles through enhancing

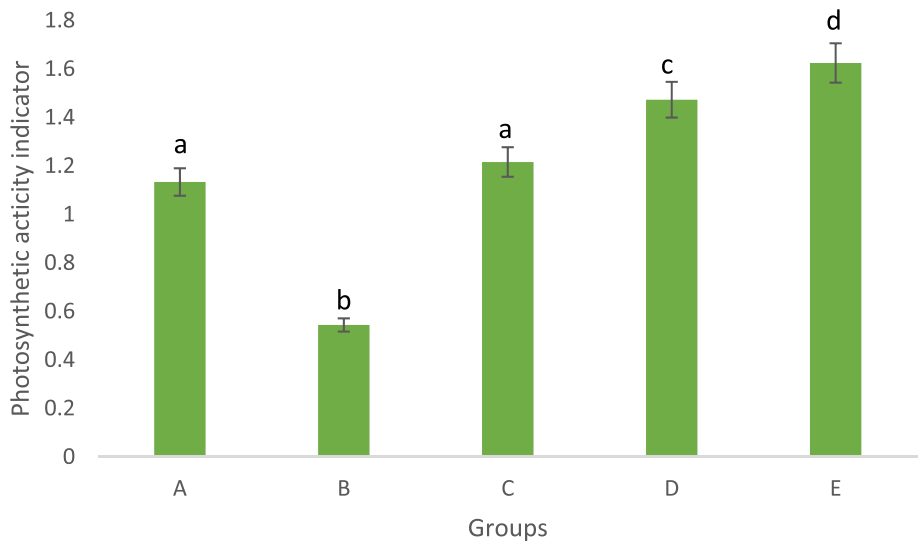


Fig. 4 Indicator of photosynthetic ability. Group: A water (control), group B: 100 mg Ca(NO3)2/kg soil, group C: 100 mg CaNPs/kg soil, group D: 75 mg CaNPs/kg soil, group E: 50 mg CaNPs/kg soil. Bars having different superscripts are significantly different ($p < 0.05$)

Table 4 Mineral element constituents ($mgkg^{-1}$) in roots percentage absorption and immobilisation of heavy metal in *M. oleifera*, planted on $Ca(NO_3)_2$ and CaNP-amended soil

Metals	Metal concentration in Root					Percentage absorption					Percentage immobilisation				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
K	79.39 ± 8.41[a]	82.49 ± 0.00[a]	83.23 ± 3.23[a,b]	86.18 ± 2.14[b,c]	89.16 ± 1.14[c]	87.05	88.28	90.41	87.52	92.21					
Na	16.05 ± 0.59[a]	15.95 ± 0.25[a,c]	11.02 ± 0.42[b]	13.60 ± 0.66[c]	12.85 ± 0.40[b]	99.09	84.08	77.86	77.42	83.85					
Ca	34.11 ± 3.23[a]	38.94 ± 1.99[b]	40.25 ± 2.91[b]	39.16 ± 2.33[b]	37.03 ± 0.07[a,b]	89.56	89.72	91.09	96.86	89.96					
Mg	23.22 ± 3.52 [a]	22.90 ± 0.99[a]	28.83 ± 7.16 [b]	24.08 ± 1.58 [a]	25.71 ± 1.61[a,b]	77.77	73.87	87.68	70.38	82.41					
Fe	28.40 ± 6.06[a]	30.39 ± 8.17[b]	34.54 ± 7.74[b]	32.36 ± 2.68[a]	33.87 ± 9.95[b]	54.82	60.64	65.84	61.29	66.26					
Mn	0.72 ± 0.01[a,b]	0.91 ± 0.00 [b]	0.93 ± 0.01 [b]	0.502 ± 0.02 [a]	0.77 ± 0.01[a,b]	43.09	56.02	57.06	38.96	68.01					
Zn	1.19 ± 0.00 [a]	1.34 ± 0.00 [b]	1.37 ± 0.00 [b]	1.35 ± 0.00 [b]	1.12 ± 0.00 [a]	92.13	97.08	98.84	83.02	81.52					
Cu	1.09 ± 0.02 [a]	0.97 ± 0.00 [a]	1.30 ± 0.01 [b]	0.94 ± 0.01 [a]	0.78 ± 0.02 [c]	88.55	66.08	90.84	49.01	51.62					
Ni	0.0223 ± 0.01[a]	0.0203 ± 0.01[a]	0.015 ± 0.01[b]	0.018 ± 0.01[a,b]	0.02 ± 0.00[a]	56.03	57.36	85.61	43.07	83.50	19.49	28.45	64.71	17.14	52.40
Cd	0.102 ± 0.00[a]	0.018 ± 0.00 [b]	0.005 ± 0.00 [c]	0.007 ± 0.00 [c]	0.005 ± 0.00[c]	20.75	16.45	4.47	6.87	4.65	28.82	84.36	84.51	84.38	85.31
Pb	0.19 ± 0.01[a]	0.103 ± 0.01 [b]	0.107 ± 0.00 [b]	0.15 ± 0.00 [a]	0.16 ± 0.00 [a]	73.09	69.07	59.33	65.60	82.48	21.71	36.13	47.24	46.03	40.40
Cr	0.027 ± 0.00[a]	0.027 ± 0.00[a]	0.021 ± 0.00[a,b]	0.022 ± 0.00[a,b]	0.018 ± 0.00[b]	14.85	17.47	14.64	15.61	14.02	6.87	21.57	28.83	26.03	34.07
As	nd	0.0020 ± 0.00	nd	nd	nd	0	12.66	0	0	0	100	14.13	100	100	100

Group A: water (control), group B: 100 mg $Ca(NO_3)_2$/kg soil, group C: 100 mg CaNPs/kg soil, group D: 75 mg CaNPs/kg soil, group E: 50 mg CaNPs/kg soil. Results of mineral elements in roots and shoot are expressed as mean ± standard deviation of three replicates. Results having different superscripts across the rows for each parameter are significantly different ($p < 0.05$). *nd*, not detected

microbial activities in soil. $Ca_3(PO_4)_2$ nanoparticles had similar nutrient-mobilising effects on rice. This has also been found in soil amended with ZnO, CuO and TiO_2 nanoparticles [2, 17, 26, 40, 51]. A significant decrease in exchangeable sodium percentage (ESP) and an increase in Ca in soil imply lower salinity of amended soils. Salinity reduces plant growth, creates an imbalance in soil-water, disrupts plant cell functions and induces metabolic disorders in plants. Nanoparticles such as nitric oxide are reportedly efficient in reducing salinity, and similarly, Ca is known to regulate the salinity of soil [3, 40, 58–60]. Correspondingly, CaNPs would be efficiently better as a soil conditioner to reduce salinity stress and provide essential minerals by maintaining K concentration.

Availability of macro- and micronutrients such as Ca, N and Fe in amended soil was higher than in raw whereas the presence of toxic heavy metals was consequently immobilised. These could be ascribed to CaNP amendment as nanoparticles are known to improve uptake of other beneficial nutrients [40]. Interestingly, as observed in EDX results, there was a mobilisation of Ag^+ by 100 mgL^{-1} CaNPs supporting possible antimicrobial potential against soil pathogens.

Germination parameters are indices to measure plant sensitivity to harsh environmental conditions, exposure to toxin and disturbance by pathogens [1, 3, 51]. Amendment with CaNPs promoted longer roots and shoots for absorption and translocation of nutrients for better *M. oleifera* yields. CaNPs hastened seed germination as observed in germination percentage by ensuring seed viability in soil and increased the number of leaves as well as vigour index to improve physiological tolerance against adverse environmental disorderliness. Vigour index can be used to access stimulatory and toxicity effects on seed germination [8, 12, 22, 25, 35]. It gives information about seed viability and its tolerance to toxicity; thus, significantly higher vigour indices recorded for *M. oleifera* planted on CaNP-amended soil (100 and 75 mgL^{-1}) indicate the stimulatory abilities of higher concentrations to influence seed germination and strengthening their physiological tolerance through

better activations of biological enzymes required for seed viability [22, 23]. Relative water contents reflect water tolerance status in plants against drought. It indicates the balance between water absorption from soil and consumption via transpiration. It is well known that nanoparticles increase plant water permeability [13, 17]. Higher relative water contents in *M. oleifera* grown on 100 and 75 mgL^{-1} CaNPs imply *M. oleifera* was more resistant against drought. These improvements in germination parameters are indications of cell promotion activities of CaNPs. This agrees with previous results of [1, 3, 8, 12, 35, 60–64]. $Ca_3(PO_4)_2$ nanoparticles similarly had a positive influence on roots, shoot lengths and some antioxidant enzymes [40]. Interaction of CaNPs with cellular components of *M. oleifera* could have resulted in improved germination as previously reported by [1].

Conversely, $Ca(NO_3)_2$-amended soil considerably reduced relative water contents possibly resulting from clogging of root pores which might be signals of cytotoxicity induced by it without providing any alleviation [13]. This was also noted by [35] that $AgNO_3$ salt solution was cytotoxic and led to a significant reduction in tomato root and shoot lengths but its nanoparticle counterpart had positive effects on these parameters.

Photosynthetic pigment contents are an expression of healthy functions of plants while carotenoids act as cellular redox buffer [1, 51, 65]. Improvements in their contents in *M. oleifera* planted on CaNP-amended soil is an attestation to enhanced enzyme activities responsible for photosynthetic mechanisms. Equally attributable to increased absorption and translocation of mineral nutrients (Fe and Mg) involved in chlorophyll formation [13, 24]. The increase was concentration-dependent. However, $Ca(NO_3)_2$ reduced carotenoid contents but had nearly similar chlorophyll contents as control. This might be ascribed to disruption in enzyme activities and water absorption capacity as noted in phytochemical and relative water contents [8, 22]. Indicator photosynthetic efficiency of CaNPs increased with decreasing concentration denoting that photosynthetic activities improved with decreasing concentration. Contrarily, $Ca(NO_3)_2$ inhibited these activities of chlorophyll by significantly reducing this ratio [13]. Carotenoids contribute to antioxidant activity; thus, its reduced content in *M. oleifera* planted on $Ca(NO_3)_2$ amended soil is an indication of reduced ability to protect against free radicals which were reported for percentage antioxidant activity. The positive influence of nanoparticles on chlorophyll pigments and carotenoids has been previously reported [1, 8, 12, 13].

Macro- and microelements are essential nutrients for various activities in plant tissues. Their presence is significantly correlated with the nutritional quality of plants. The increase in absorption and translocation rates of K, Ca, Fe, Mg, Zn and Cu obtained in this study suggest *M. oleifera* planted on amended soil are better sources of these nutrients for human consumption [43]. This is in addition to higher immobilisation rates of heavy metals. Ca, Mg, Fe, Zn and Mn play prominent roles in bone development, glucose absorption, and regulation of blood and act as co-factors for enzymes. They play vital roles in the cation exchange capacity of roots leading to more absorption of essential nutrients such as N that may contribute to higher protein contents in plant parts [26, 38, 64]. $Ca_3(PO_4)_2$ nanoparticles have been reported to control pathogen infestation of *Zizyphus mauritiana* and *Citrus tankan* and assisted in the uptake of minerals that are required for metabolic activities in plants [7, 40, 58, 60]. Improvements in mineral contents in *M. oleifera* by CaNPs imply that CaNPs can act as nano-fertilisers to enhance upward translocation of minerals to plants as it has been previously recorded that nanoparticles led to increased contents of these minerals in plants [1, 13, 14, 32, 36, 66, 67]. Additionally, large surface area and high penetration potentials of macronutrient nanofertilisers ensure efficiency in the delivery of nutrients to plant as have been demonstrated in studies reported for hydroxyapatite (P), nano-enabled urea (N) and $Ca(PO_4)_2$ (Ca, P) nanoparticles with improved macronutrient quantities in plant and enhanced plant metabolisms [1, 39, 40].

Heavy metals such as Cd, Cr, Pb and As do not have known biological values and are extremely toxic [25]. The presence of heavy metals in *M. oleifera* planted on CaNP-amended soil greatly reduced with total immobilisation of As. This might be ascribed to the surface charges of CaNPs (pH_{pzc}) and soil pH. The pH_{pzc} of CaNPs was lower than soil pH (pH_{pzc} < pH), therefore would be anionic and would be available for adsorption of cations. Results of immobilisation of heavy metals with CaNPs in this study are consistent with previously reported results of metal nanoparticles by [8, 46, 68]. Nanoparticles have good adsorption capacities stemming from their morphology, surface charge, reactivity and size. Hence, their addition to soil was expected to improve the phytoremediation potential of *M. oleifera* and the adsorption capacity of the soil.

5 Conclusion

This study has reported the interplay between the application of CaNPs, soil fertility and modulatory influence on *M. oleifera*. CaNPs enlarged soil pores, improved soil fertility by increasing nitrogen contents and cation exchange capacity with a concomitant reduction in Na activities. Remarkable improvements in both macro- and micronutrient levels and immobilisation of heavy metals were recorded. Considerable promotions in growth parameters, physiological tolerance, higher translocation

rates of essential nutrients and better indicators of efficiency of photosynthetic activities are confirmations of the phytomodulatory abilities of CaNPs.

Abbreviations

A: Group A (water); B: 100 mg $Ca(NO_3)_2$/kg soil (group B); C: 100 mg CaNPs/kg soil (group C); CEC: Cation exchange capacity; CaNPs: Calcium nanoparticles; D: 75 mg CaNPs/kg soil (group D); E: 50 mg CaNPs/kg soil (group E); ESP: Exchangeable sodium percentage; ICP-OES: Inductively coupled plasma with optical emission spectrometer; *M. oleifera*: *Moringa oleifera*; pHpzc: pH point of zero charge; SEM-EDX: Scanning electron microscope-energy dispersive x-ray

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Declarations

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Authors' contributions

LA synthesised and characterised calcium nanoparticles using the pod extract of *C. nitida*. AL coordinated the planting, soil analysis and manuscript write-up. ARA and AAE handled elemental analysis. All authors have read and approved the manuscript

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