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Assessment and nematicidal activity of Cu/Fe and Zn/Fe bimetallic nanoparticles against root-knot nematode in beetroot and cabbage

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This research intended to evaluate the potency of bimetallic nanoparticles (BMNPs) and *Cyclotella meneghiniana* on two pivotal plant parasitic nematodes (PPN). A preliminary in vitro experiment was conducted to screen the effectiveness of both control materials against *Meloidogyne incognita* and *Scutellonema bradys*. *C. meneghiniana* displayed very weak activity on hatching inhibition of *M. incognita* eggs and mortality of *S. bradys* individuals. Potent action was recorded in BMNPs on *M. incognita* juvenile mortality and egg hatch inhibition. The suppressive action of BMNPs on *M. incognita* was assessed in vivo on infected beetroot (*Beta vulgaris* L.) and cabbage (*Brassica oleracea* L.) in a field experiment laid out in randomised complete block design. Activity of BMNPs was concentration dependent. Copper/iron nanoparticles (Cu/Fe NPs) enhanced yield and vegetative parameters of both plants. Cu/Fe exhibited higher antioxidant activity in comparison with Zn/Fe, thus explaining the potency of Cu/Fe on most parameters evaluated. High resolution transmission electron microscopy (HRTEM) confirmed particle size of ZnO/Fe₂O₃ NPs and Fe₂O₃/CuO NPs to be 12.5 nm and 15.4 nm, respectively. High angle annular dark-field (HAADF STEM) identified the compositional distribution of each element, while selected area diffraction (SAD STEM) confirmed the crystalline nature of BMNPs. Bimetallic NPs are a good proposal for *M. incognita* management on beetroot and cabbage plants.

Keywords *Cyclotella meneghiniana*, Bimetallic nanoparticles, Antioxidant, Nematodes

A dominant biotic factor that minimises and impairs quality of crops worldwide is plant parasitic nematodes (PPNs). The root knot nematode comprises about 100 species, which pose a huge threat among other PPNs owing to their extensive host range¹. *Meloidogyne* species are globally widespread and are particularly problematic on vegetable and tuber crops. *Meloidogyne* spp produce high amounts of inoculum after one cycle of infection, which often causes stunting and may result in the death of crops^{2,3}. *Scutellonema bradys* occurs in tropical and sub-tropical areas of the world, with high statistics in Africa^{4,5} where it has high economic significance on tuber crops.

In order to realise high level yield and first grade harvest, synthetic nematicides are added extensively to cultivable lands for *M. incognita* and *S. bradys* management. Nematicides are highly effective in nematode management but with side effects, such as resistant strain evolution, human health disorders, soil microbe alteration and ecosystem modification^{6,7}. Pesticides are degraded enzymatically and they are incrementally metabolized, thus aiding their mobility in the environment⁶. Of great concern is improvement on synthetic

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nematicides as a sole means of nematode control using substances with minimal ecosystem toxicity. A consideration in this regard is the use of microalgae and nanoparticles.

Microalgae serve as a biological control agent in nematode management. They are good sources of antioxidants and carotenoids⁸. Quite a number of algae, diatoms and cyanobacteria have been reported to exhibit antiviral, antimicrobial and potent nematocidal action on *M. incognita*^{9–12}. *Cyclotella meneghiniana* f. *plana* (Fricke) Hustedt, 1928, a member of the class Bacillariophyceae and family Stephanodiscaceae^{13,14}, is a freshwater diatom species, which also exists in brackish water and sediments. It has been discovered and described in Nigerian water bodies¹⁵, particularly in fresh and at times brackish water during rainy seasons. *C. meneghiniana* is a good producer of brassicasterol, stigmaterol, campesterol, β -sitosterol and ergosterol, which are known to have cytotoxic properties^{16,17}.

Application of nanoparticles (NPs) in crop disease management offers a glimmer of hope in moderating the disturbing effects of PPNs on crops. Nanoparticles could be made from materials like carbon, metals, dendrimers, or composites. Several metal oxide NPs have exhibited potency in the management of PPNs^{18–21}. However, bimetallic NPs are reported to be more potent than monometallic NPs²².

Beetroots (*Beta vulgaris* L.) and Cabbage (*Brassica oleracea* L.) are vegetables of considerable value to human dietary requirements. Beetroots are abundant source of carotenoids, flavonoids, phenolic and ascorbic acids. Betalains, a secondary metabolite that is endowed with an array of biological actions coupled with potential of likely disease inhibition and management, abounds in beets^{23,24}. Similarly, cabbage contains folate, vitamins B6, C and K^{25,26}, which are excellent dietary requirements.

Meloidogyne incognita, a plant parasite, produces the characteristic root-knots or galls on beets and cabbage roots, thus severely limiting yield. Harvested beets are disfigured with galls, resulting in lessened quality and marketability. Consequently, farmers' productivity and profit are marginal in both crops^{27–29}. Reports on usage of bimetallic NPs in the management of PPNs in broad terms and *M. incognita* specifically are very scarce in literature. Hence, the objective of this research was to assess the potency of *Cyclotella meneghiniana* and bimetallic NPs, vis-à-vis zinc/iron and copper/iron bimetallics, in laboratory experiments on *M. incognita* and *S. bradys*. The field experiment seeks to establish the role of bimetal NP combination in the management of *M. incognita* on two highly susceptible vegetables - *Beta vulgaris* L. and *Brassica oleracea* L.

Materials and methods

Plant material collection, identification and authentication

Khaya senegalensis leaves were collected with permission from Federal University of Lafia, Nasarawa State, Nigeria. Plant identification was done at Department of Plant Science and Biotechnology Herbarium. The plant was assigned voucher number 064 and specimen was deposited in the herbarium of University of Lafia, Nigeria after identification by Mr Markus Musa. The collection and use of the plant material complied with all relevant institutional, national, and international guidelines and legislation. *Khaya senegalensis* is not listed as an endangered or protected species under the IUCN Red List or the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). This research adheres to the IUCN Policy Statement on Research Involving Species at Risk of Extinction and complies with the principles of the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Plant extract preparation

Khaya senegalensis leaves (Fig. 1) were rinsed using double-distilled water to eliminate dust and dirt and allowed to air dry at ambient temperature until crisp dry. The moisture free leaves were blended into fine powder using a laboratory Waring® blender. Following the procedure of David et al.³⁰, leaf powder weighing 10 g was suspended in 100 mL double-distilled water for 3 days with intermittent agitation. The solution obtained was filtered through a layer of Whatman No. 1 filter paper. The extract was stored at 4 °C until required.

Fe₂O₃/CuO and Fe₂O₃/ZnO NPs synthesis

Fe₂O₃/CuO NPs were synthesized using a mixture of 5 mL each of 0.2 M CuSO₄·5H₂O and 0.2 M Fe(NO₃)₃·9H₂O introduced into 90 mL of distilled water in a 250 mL beaker with constant stirring²¹. Then, 10 mL of *K. senegalensis* leaf extract was added dropwise to the reaction mixture with continuous stirring. The extract acted as both a reducing agent, facilitating the conversion of metal ions to their corresponding nanoparticles, and a stabilizing agent, preventing agglomeration by capping the nanoparticle surfaces. The colour changes of the reaction mixture from pale yellow to brown indicated that Cu/Fe nanocomposite had been synthesized (Fig. 1). The reaction was allowed to continue for an additional 30 min. The synthesized Fe₂O₃/CuO NPs were centrifuged at 10,000 rpm for 30 min and rinsed three times using distilled water/ethanol for the removal of impurities. Fe₂O₃/CuO NPs pellets were then oven dried at 60 °C for 12 h.

The synthesis of Fe₂O₃/ZnO NPs also followed the procedure of Fabiyi et al.²¹, except that ZnSO₄·5H₂O replaced CuSO₄·5H₂O in the same concentration and volume. When 10 mL of *K. senegalensis* leaf extract was added in drops while stirring vigorously, the reaction mixture colour turned to dark grey from colourless, signalling Fe₂O₃/ZnO NPs production (Fig. 1). This reaction also continued for 30 min. The Zn/FeNPs were separated through centrifugation at 10,000 rpm for 30 min, rinsed using distilled water/ethanol to remove impurities, and oven dried at 60 °C for 12 h. Both NPs were later ground into fine powder using a porcelain laboratory mortar and pestle.

Characterization of bimetallic nanoparticles

The wavelength absorption of the synthesized bimetallic oxide NPs was examined using scanning UV/Visible spectrophotometer 240 V Jenway 741,501. The shape and size of synthesized NPs were assessed using electron microscope, model FEI Titan 80–300. HAADF-TEM, SAD and FTIR were performed using a Nicolet Summit

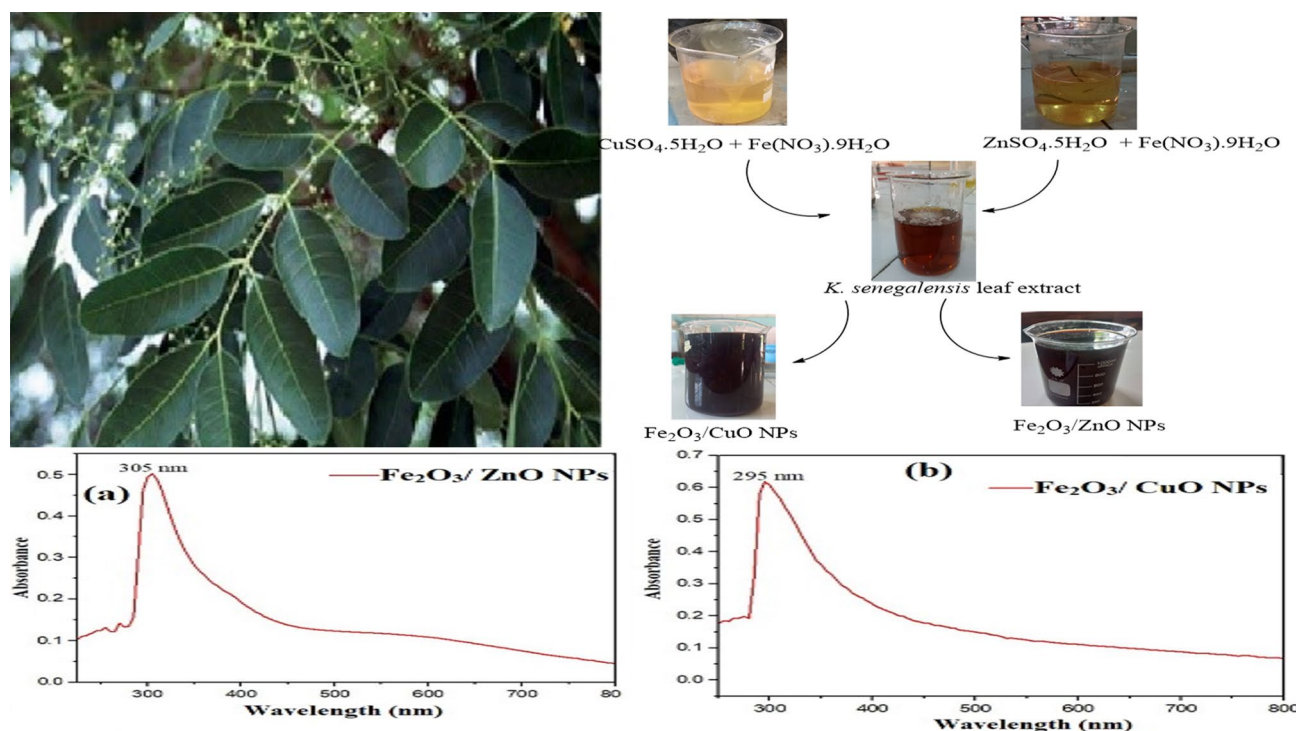


Fig. 1. (Top left): *Khaya senegalensis* leaves; (Top right): Schematic synthesis of $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs and $\text{Fe}_2\text{O}_3/\text{CuO}$ NPs. (Bottom): (a) UV spectra of $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs (b) $\text{Fe}_2\text{O}_3/\text{CuO}$ NPs.

FTIR spectrometer to establish the surface functionalities of synthesized bimetallic NPs. Nicolet Summit FTIR spectrometer was calibrated and optimized for the desired spectral range ($4000\text{--}500\text{ cm}^{-1}$). An appropriate ATR crystal (zinc selenide) for the analysis was selected and ensured to be clean and free from contaminants.

Antioxidant analysis

The antioxidant properties of Zn/Fe and Cu/Fe bimetallic nanoparticles (NPs) were determined using ascorbic acid as the standard and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) as the test reagent. The ABTS radical cation ($\text{ABTS}^{+\bullet}$) was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) in double-distilled water at a 1:9 (v/v) ratio and allowing the mixture to stand in the dark at room temperature for 16 h before use. For the assay, 190 μl of methanol was added to a microtiter plate, followed by the addition of 10 $\mu\text{g}/\text{ml}$ of the sample. Sample dilutions were prepared by mixing 10 $\mu\text{g}/\text{ml}$ of sample with 990 μl of ABTS working solution, 20 $\mu\text{g}/\text{ml}$ of sample with 980 μl ABTS, 50 μl with 950 μl ABTS, 100 μl with 900 μl ABTS, and 150 μl with 850 μl ABTS. Absorbance was measured at 735 nm using a spectrophotometer BioRad model 680, Japan. The control (A0), consisting of ABTS without any sample, was measured. After 6 min of initial mixing, absorbance of the sample (A6) was recorded. Each sample was tested in triplicate. The percentage of ABTS radical scavenging activity was calculated using the formula: % ABTS SCAVENGING = $[(A0 - A6) \div A0] \times 100$.

Preparation of *Cyclotella meneghiniana*

The algae culture was obtained from Dr T.A. Adesalu, University of Lagos, Nigeria. Microalgae cultures were first centrifuged to separate algae from the media and remove any dirt or impurities, then the microalgae were washed in distilled water and the concentrated microalgae were freeze-dried following the procedure of¹⁵. The microalgae were first frozen at a temperature between -80 and $-196\text{ }^\circ\text{C}$ (at St. Cloud State University, USA). The frozen microalga was placed in a vacuum chamber for primary drying, where the temperature was increased to a point where water could sublimate, hence removing the majority of the water content. Immediately after, the microalga was subjected to secondary drying, and the temperature was increased once more to remove any remaining water content. The microalga was entirely dry and stored for further use.

In vitro nematocidal assay

M. incognita eggs were obtained from previously identified single population cultures at the Department of Crop Protection and Environmental Biology, University of Ibadan. Galled roots of *Celosia argentea* plants served as inoculum source for the laboratory assay. Steps for inoculum preparation followed NaOCl procedure of Hussey and Baker³¹. The extracted eggs were divided into two batches, and one batch was allowed to hatch at room temperature on the laboratory bench. For *S. bradys* inoculum, infected yam tubers were visually assessed in the market and taken to the laboratory for extraction³². In the laboratory, the tuber outer cortex was peeled off with the aid of a kitchen knife up to about 2 mm deep. The peels were finely chopped and set up for extraction

following modified Baermann protocol³³. *S. bradys* juvenile and adult suspension was collected after 48 h. A micro-Pipette was used to dispense 0.1 mL nematode extracts containing 50 eggs/individual into 2 cm microwells. *C. meneghiniana* (100 g) was suspended for extraction in 300 mL distilled water, while bimetals and Furadan were equally dissolved in 300 mL distilled water at 100 g each to make a stock solution for individual treatments. The concentrations were further diluted into 60 and 30% concentrations from which two 2 mL of each was dispensed into microwells containing *M. incognita* eggs and juveniles, and *S. bradys* mixed stage individuals. Data were collected on egg hatch inhibition and mortality of nematode juveniles.

Field experiment

The research design consisted of four treatments, each with four levels in a randomized complete block design in five replicates. Plot sizes were 2×5 m ($n = 80$) made after ploughing and harrowing for each of the test crops, thus totalling 160 plots. Seeds of beetroots and cabbage were sown directly at a spacing of 25 cm and 40 cm intra and inter rows, respectively. *M. incognita* juveniles (J_2) extracted from heavily galled roots of *C. argentea* served as source of inoculum. A week after sprouting, beetroot and cabbage plantlets were inoculated with J_2 at 50/mL in 100 mL suspension. Treatment application was done at two weeks post inoculation. Furadan was applied at 2.0, 1.5 and 1.0 kg/a.i/ha while the bimetal NPs were at 100, 75 and 50 ppm, and NPK fertilizer was at equivalent of 20, 40 and 60 kg/ha. Necessary agronomic practices for the two vegetables were followed during the growth period. Assessment of beets and cabbage root damage expressed as gall index, followed the scale established by Bridge and Page³⁴.

Statistical analysis

Data were analysed with R software version 3.1.0³⁵. Time was considered the main factor and Tukey's HSD was used to separate means ($p \leq 0.05$).

Results

UV-Visible spectroscopy

Absorbance in the UV-Vis region was demonstrated by bimetallic NPs; therefore, UV-Vis spectrophotometric examination provides immediate foundational evidence of NP production. The UV-Vis spectrum was obtained to confirm the synthesis of $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs. As shown in Fig. 1a, the peak at 305 nm for $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs falls within the absorbance ranges of the individual components: ZnO typically absorbs between 300 and 320 nm, while Fe_2O_3 shows peaks between 270 and 320 nm, depending on crystallinity and synthesis method. The merged and slightly shifted peak at 305 nm indicates the formation of a bimetallic composite rather than a physical mixture, consistent with previous reports on Fe-Zn oxide nanostructures^{36,37}. This shift suggests electronic interaction and confirms successful bimetallic nanoparticle synthesis. Figure 1b displays the UV-vis spectra of $\text{Fe}_2\text{O}_3/\text{CuONPs}$. According to literature, CuO nanoparticles exhibit absorbance between ~250 and 325 nm (e.g., 250 nm peak with ~350 nm shoulder or prominent 325 nm SPR), while Fe_2O_3 nanoparticles absorb broadly between ~320 and 420 nm. In our $\text{Fe}_2\text{O}_3/\text{CuO}$ composite, the UV-Vis peak shifts to 295 nm (versus 325 nm for monometallic Fe_2O_3), indicating interaction between the oxides and formation of a hybrid nanostructure.

High resolution transmission electron microscopy (HRTEM) analysis

The HRTEM representation of $\text{Fe}_2\text{O}_3/\text{ZnONPs}$ and $\text{Fe}_2\text{O}_3/\text{CuO}$ NPs is shown in Figs. 2a & b top. Further studies were conducted on individual particles of $\text{Fe}_2\text{O}_3/\text{ZnONPs}$ using HRTEM to more clearly clarify size plus shape of the nanoparticles. Both metallic oxide NPs were spherical in size with good dispersion (Fig. 2a and b top). Average particle size of $\text{ZnO}/\text{Fe}_2\text{O}_3$ NPs and $\text{Fe}_2\text{O}_3/\text{CuONPs}$ was 12.5 nm and 15.4 nm, respectively. The metallic oxide nanoparticles are widely distributed, and although iron nanoparticles are very reactive, they readily oxidize in an open atmosphere and have a tendency to agglomerate³⁸.

FTIR analysis

For $\text{Fe}_2\text{O}_3/\text{ZnONPs}$, an intense broad band ranging from $3500\text{--}3000\text{ cm}^{-1}$ (Fig. 2a bottom) was observed and conforms with OH group's stretching vibration, whereas presence of water molecules in the samples caused the H-O-H group's bending vibrations to emerge at 1600 cm^{-1} ³⁹. The weak band observed at 1695 cm^{-1} is related to stretching vibration of C=C. Peak at 1339 cm^{-1} is due to the presence of C-H alkane. The peak at 1217 cm^{-1} may correspond to C-N stretching vibrations, while 763 cm^{-1} is tentatively assigned to aromatic C-H bending (phenyl group), consistent with reports by Margoshes & Fassel⁴⁰, and Rastgar et al.⁴¹. These assignments suggest the presence of organic compounds from the plant extract acting as stabilizing agents on the nanoparticle surface. Spectrum of parent magnetite NPs depicts strong peaks at a lower frequency of 504 cm^{-1} by reason of Fe-O functional group⁴². The characteristic peak at 503 cm^{-1} and below is assigned to Zn-O bands. A similar observation was reported by Gautam et al.³⁷. From FTIR spectra of $\text{Fe}_2\text{O}_3/\text{CuO}$ NPs (Fig. 2b bottom), the peaks around 3691.5 ; 2965.6 ; 1605.9 , and 1056.8 cm^{-1} are attributed to the O-H, C=C, C=O, and C-O that are fundamental units for many organic biomolecules. The results obtained show evidence of surface functionalization due to constituents of phenolic compounds, which affirms ability of extract to reduce $\text{Fe}_2\text{O}_3/\text{CuO}$, hence, bimetallic nanoparticles are synthesized with this strong indicator. The peaks found within the range $800\text{--}600\text{ cm}^{-1}$ represent Fe-O and Cu-O of the $\text{Fe}_2\text{O}_3/\text{CuO}$ bimetallic NPs⁴³.

HAADF STEM analysis

High angle annular dark-field (z-contrast imaging) in Fig. 3a depicts the STEM image of $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs with lighter components in the shell and rather heavier elements at the core, demonstrating the enhanced Z contrast provided by an HAADF detector. The results clearly state the distribution of $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs⁴⁴. The HAADF

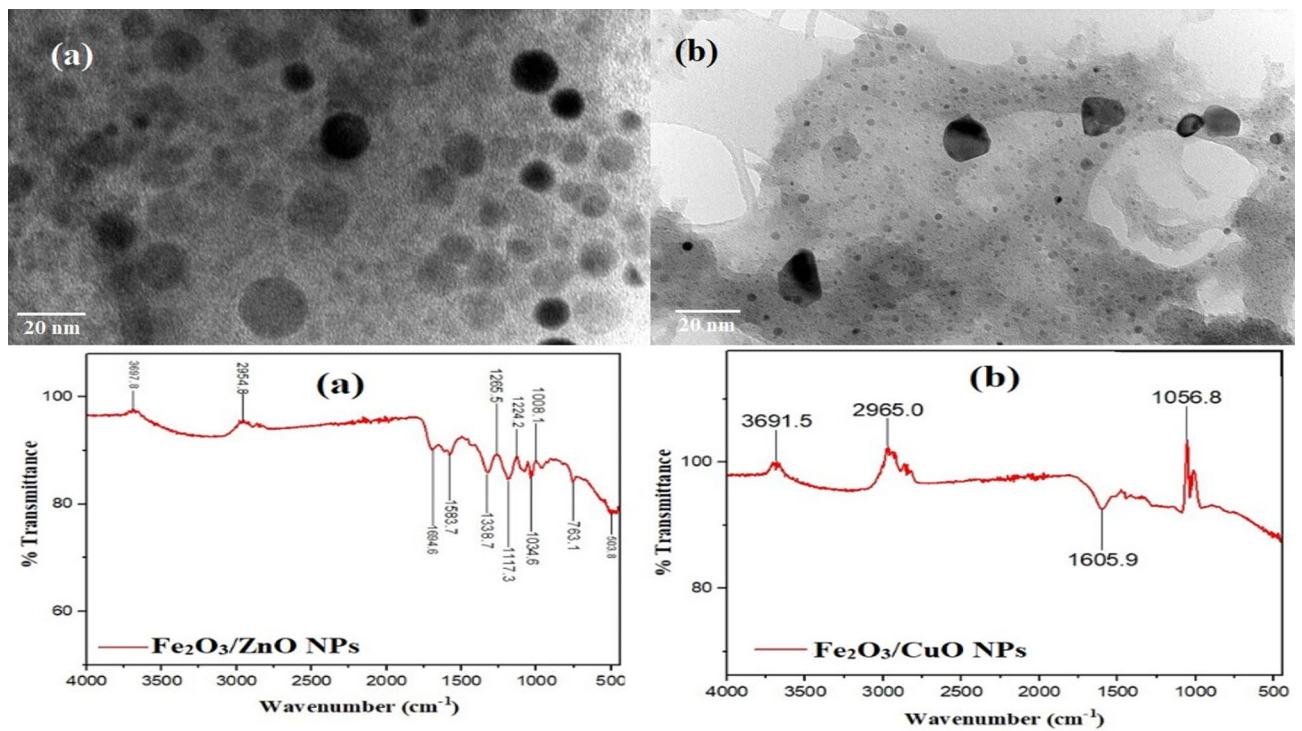


Fig. 2. (Top): HRTEM images of (a) $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs and (b) $\text{Fe}_2\text{O}_3/\text{CuO}$ NPs. (Bottom): (a) FTIR spectra of $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs (b) $\text{Fe}_2\text{O}_3/\text{CuO}$ NPs.

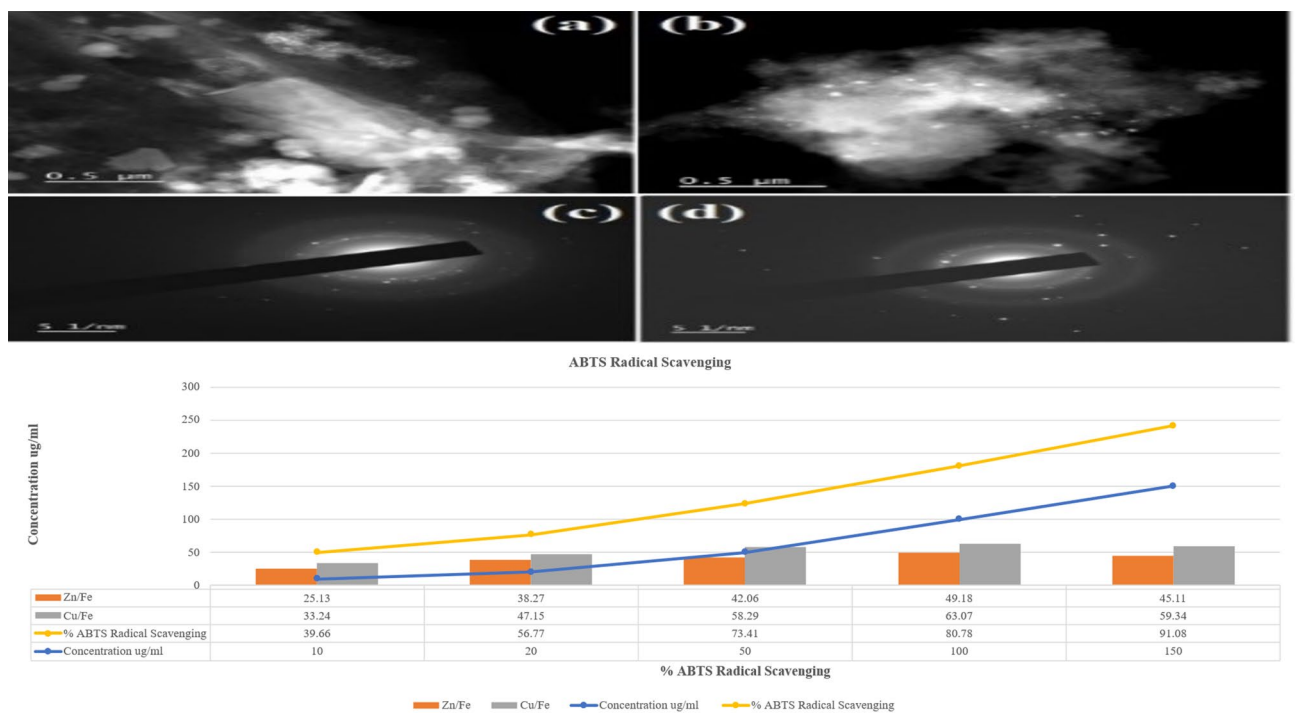


Fig. 3. Top HAADF STEM images of (a) $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs (b) $\text{Fe}_2\text{O}_3/\text{CuO}$ NPs and SAD STEM images of (c) $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs (d) $\text{Fe}_2\text{O}_3/\text{CuO}$ NPs. Bottom radical scavenging activity of bimetallic NPs.

STEM image of $\text{Fe}_2\text{O}_3/\text{CuO}$ NPs showed the distribution of CuO and Fe_2O_3 nanoparticles. It is observed that the CuO- Fe_2O_3 bimetallic clusters with the compositional distribution of each element (Fig. 3b)⁴⁵.

SAD STEM analysis

Figure 3c depicts selected area diffraction (SAD) conducted on dissimilar particle of $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs. It displays coaxial diffractive rings accompanied by bright spots. It was detected that all particles obtained were crystalline⁴⁶. The bright circular rings correspond to the planes, which are due to the formation of the fcc structure of the metallic $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs. More intense diffuse rings are seen in the SAD pattern of $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs, which suggests the formation of small particle size, which corresponds to the result obtained from the TEM analysis. The SAD patterns confirm the presence of $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs and their high purity due to the lack of interferences from other ferric and ferrous salts [47]. The selected area diffraction of $\text{Fe}_2\text{O}_3/\text{CuO}$ NPs (Fig. 3d) showed concentric diffraction rings in company of some bright spots. It was observed that all the particles that were obtained were crystalline⁴⁸. The planes are due to the formation of fcc structure of metallic $\text{Fe}_2\text{O}_3/\text{CuO}$ NPs. The synthesis of well-dispersed $\text{Fe}_2\text{O}_3/\text{CuO}$ NPs and $\text{Fe}_2\text{O}_3/\text{ZnO}$ NPs is confirmed by the SAD pattern.

Antioxidant activity

Antioxidant testing was conducted to assess the biological relevance of the synthesized bimetallic nanoparticles, particularly their potential to neutralize free radicals and mitigate oxidative stress. The ABTS assay was used to quantify their radical scavenging capacity relative to ascorbic acid. (Table 1; Fig. 3 bottom). In addition to biological evaluation, antioxidant compounds in the *Khaya senegalensis* extract likely contributed to nanoparticle formation and stability by acting as reducing and capping agents during synthesis. Phenolic compounds and flavonoids present in the extract can donate electrons to reduce metal ions and also adsorb onto the NP surface, preventing agglomeration⁴⁹.

Khaya senegalensis bimetallic nanoparticles (NPs) antioxidant properties were studied through the ABTS radical scavenging assay, which used ascorbic acid as its reference compound. It was observed that percentage scavenging ability of Zn/Fe and Cu/Fe bimetallic nanoparticles increased as their concentration levels (10–150 $\mu\text{g}/\text{mL}$) rose. The scavenging ability of ascorbic acid rises progressively with increasing concentrations and achieves its highest value of 91.08% at 150 $\mu\text{g}/\text{mL}$. Laboratory tests demonstrated that ascorbic acid offers a powerful ability to neutralize radicals, which proves its usefulness as a reference material. The antioxidant efficiency of Zn/Fe bimetallic NPs increases slightly from 25.13 to 49.18% when the dosage increases from 10 to 100 $\mu\text{g}/\text{mL}$, then decreases marginally to 45.11% at 150 $\mu\text{g}/\text{mL}$. The experimentation indicates that antioxidant strength falls between the results of ascorbic acid and Zn/Fe bimetallic NPs. Scavenging capability reaches its maximum when using Cu/Fe NPs at 100 $\mu\text{g}/\text{mL}$, with 63.07% until it decreases to 59.34% at 150 $\mu\text{g}/\text{mL}$. The observed decline in scavenging activity at 150 $\mu\text{g}/\text{mL}$ for the NPs may be attributed to aggregation of nanoparticles at higher concentrations, which reduces their effective surface area and interaction with free radicals. A similar phenomenon has been reported in plant-mediated nanoparticles, where excess concentration leads to reduced dispersion and decreased radical scavenging efficiency due to nanoparticle aggregation^{49,50}.

Juvenile mortality and egg hatch

Figures 4, 5, and 6 show response of *M. incognita* juveniles/eggs and *Scutellonema bradys* to the treatments at various concentrations of application. There was no mortality in the control experiment throughout seven day period of exposure of juveniles to treatments. Mortality was significantly high in the bimetallic NP treatments. Cu/FeNPs at 60% concentration provided the highest juvenile mortality, followed by Zn/FeNPs (60%), Cu/Fe

Samples	Concentration ($\mu\text{g}/\text{ml}$)	% ABTS Scavenging
Ascorbic Acid (Standard)	10	39.66 ^k
	20	56.77 ^f
	50	73.41 ^c
	100	80.78 ^b
	150	91.08 ^a
Zn/Fe Bimetallic NPs	10	25.13 ^m
	20	38.27 ^k
	50	42.06 ^j
	100	49.18 ^g
	150	45.11 ⁱ
Cu/Fe Bimetallic NPs	10	33.24 ^j
	20	47.15 ^h
	50	58.29 ^{ef}
	100	63.07 ^d
	150	59.34 ^e

Table 1. Antioxidant activity of *Khaya senegalensis* bimetallic nanoparticles. Means followed by the same letter are not significantly different at $p < 0.05$ using the new DMRT. DMRT = Duncan's multiple range test. Each value is a mean of three replicates.

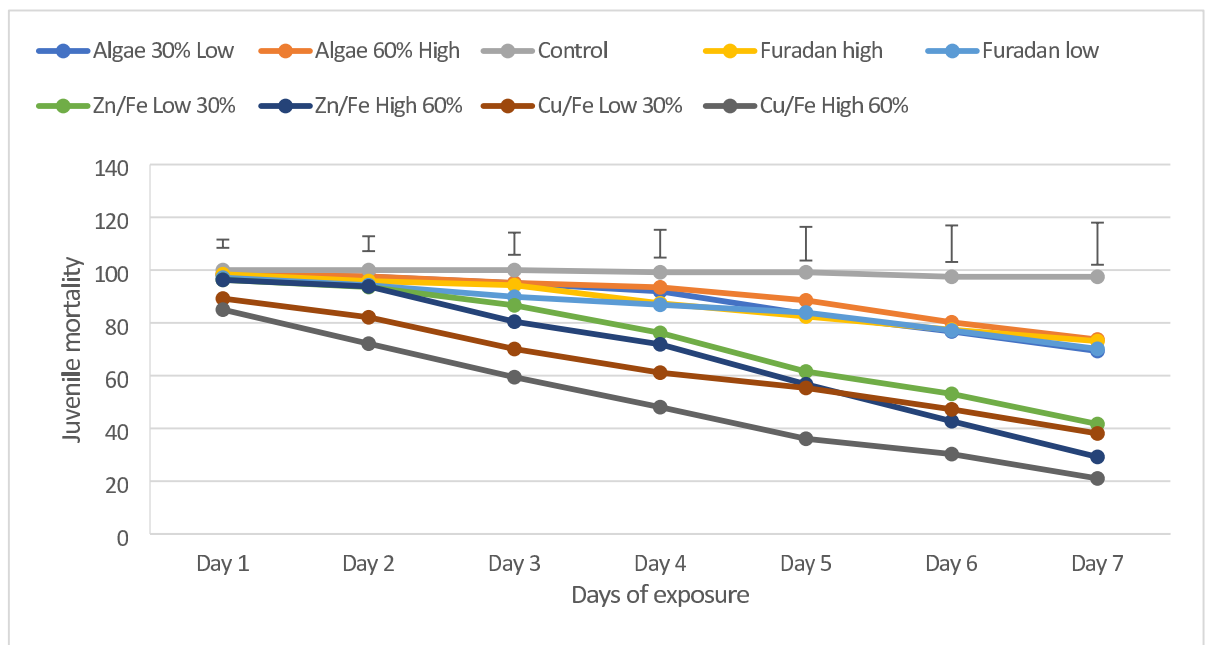


Fig. 4. Effect of treatment materials on mortality of *M. incognita* juveniles. Error bars represent standard error of means (SEM) comparing treatments and with days of exposure.

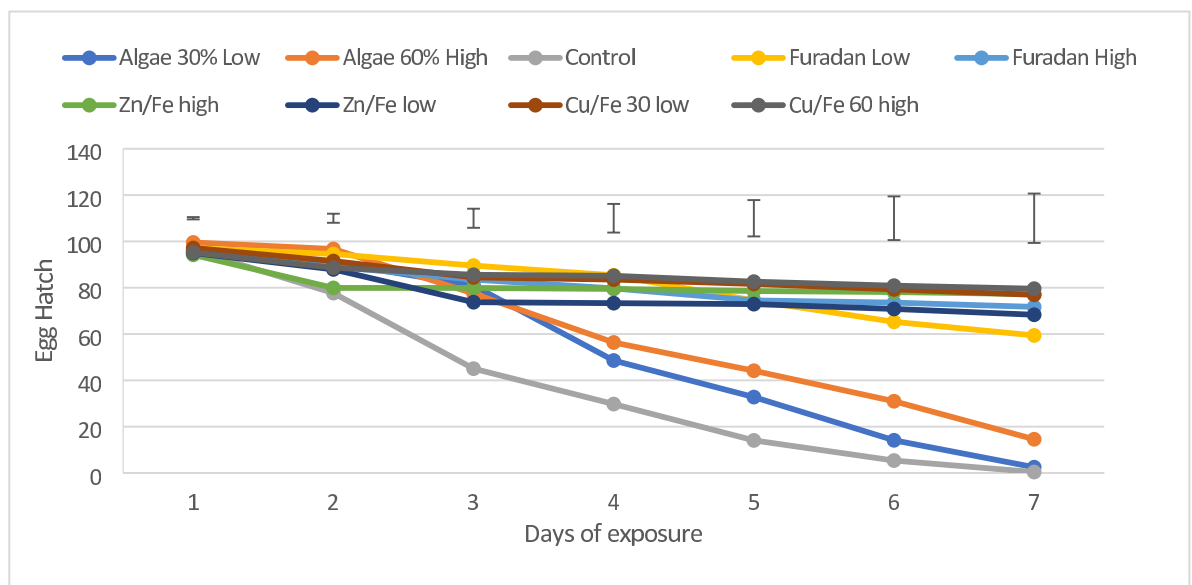


Fig. 5. Effect of treatments on hatching of *M. incognita* eggs. Error bars represent standard error of means (SEM) comparing treatments with days of exposure.

(30%) and Zn/Fe (30%). Algae and furadan at high and low concentrations exhibited minimal juvenile mortality in comparison with the bimetal NPs (Fig. 4). The order of egg hatch inhibition was Cu/FeNPs (60%) > Cu/Fe (30%) > furadan high (60) > Zn/Fe (30%) > furadan (30%), while algae at 60 and 30% had significantly lower egg hatch inhibition (Fig. 5). Likewise, high mortality of *Scutellonema bradys* individuals was recorded in Cu/FeNPs at 60%. There was a significant difference between Cu/FeNPs at 60% and Cu/FeNPs at 30% while an overlap was seen in the effect of other treatments (Fig. 6).

Field experiment

M. incognita reproductive parameters were significantly reduced in cabbage plants treated with Cu/Fe NPs in comparison with Zn/Fe NPs. Furadan was not as effective as the bimetal NPs on juvenile population and soil nematode in 200 cm³ soil, while nematode population was high in NPK treatments (Fig. 7). Root

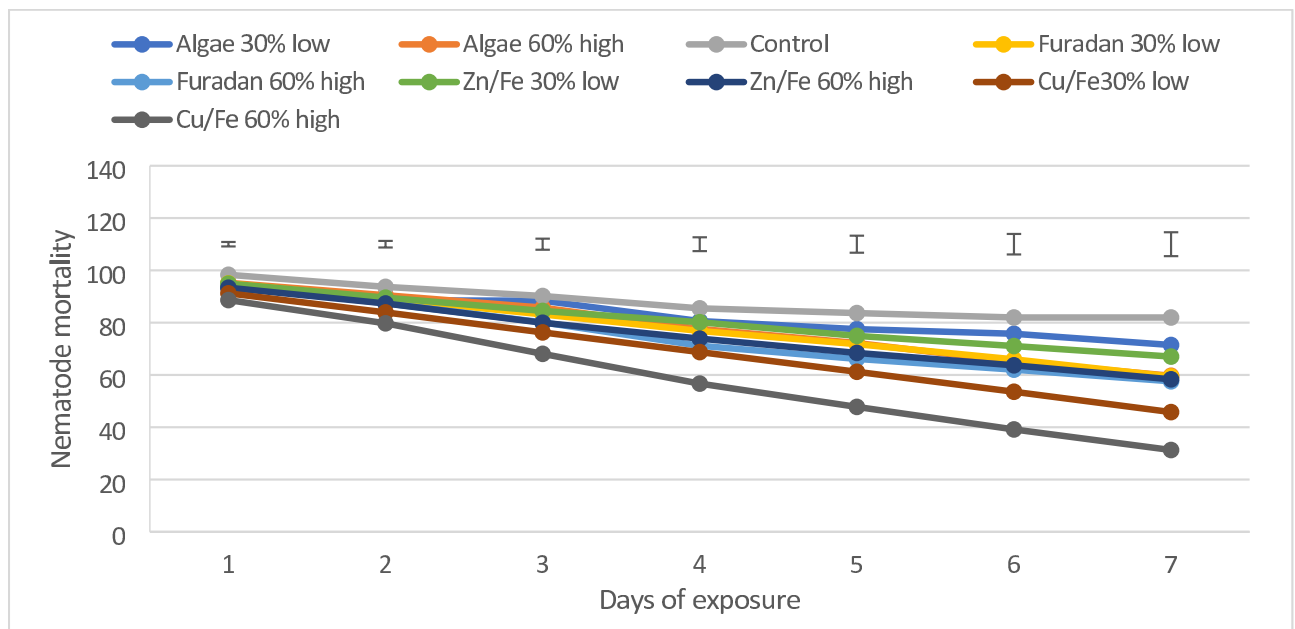


Fig. 6. Effect of treatment materials on mortality of *S. bradys* individuals. Error bars represent standard error of means (SEM) comparing treatments with days of exposure.

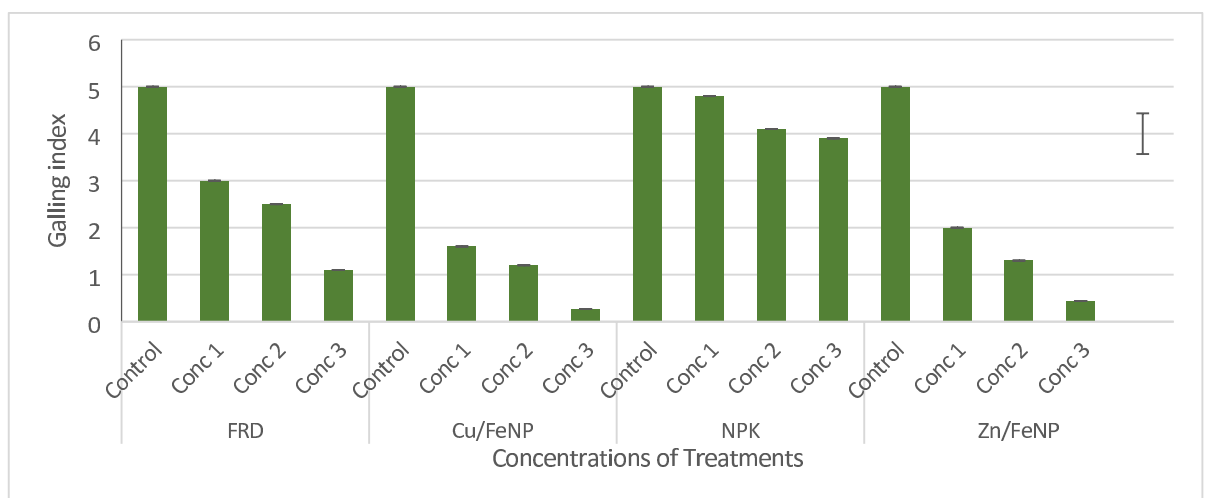


Fig. 7. Effect of treatments and concentration on galling index of cabbage plants. Error bar represents standard error of means (SEM) comparing treatments and concentration on galling index.

gall development was significantly reduced in BMNPs treated plants, as opposed to Furadan and NPK. The highest galling index was 2 in Zn/Fe NPs, while it was less than 2 in Cu/Fe NPs. Furadan recorded 3, while NPK had above 5, indicating excessive galling (Fig. 8). Yield was higher in Cu/Fe NP and Zn/Fe NP; it was comparatively low in Furadan, while the lowest yield was in NPK treatments (Fig. 9). Vegetative growth was significantly improved with the application of bimetal NPs as control material for cabbage plants infected with *M. incognita*. Cu/Fe NPs were significantly more promising at the highest concentration used (Fig. 10). Effect of bimetallic NP treatments on *M. incognita* infected beetroot plants is presented in Figs. 11 and 12. 13 and 14. Taller plants were recorded in bimetal treated plants, with Cu/Fe NP treated plants having the tallest plants (Fig. 11). Yield was also higher in Cu/Fe NP treated plants as opposed to Furadan and NPK (Fig. 12). The order of reduction by treatments of nematode population in 200 cm³ soil and 10 g root sample of beetroot plants is Zn/Fe NP > Cu/Fe NP > Furadan > NPK (Fig. 13). Gall index reduction equally followed the same pattern (Fig. 14). The concentration of all treatments had significant effect on the parameters evaluated. Higher concentration produced higher vegetative growth and yield in cabbage and beetroot. Soil and root nematodes were equally significantly reduced with higher concentrations of treatment application.

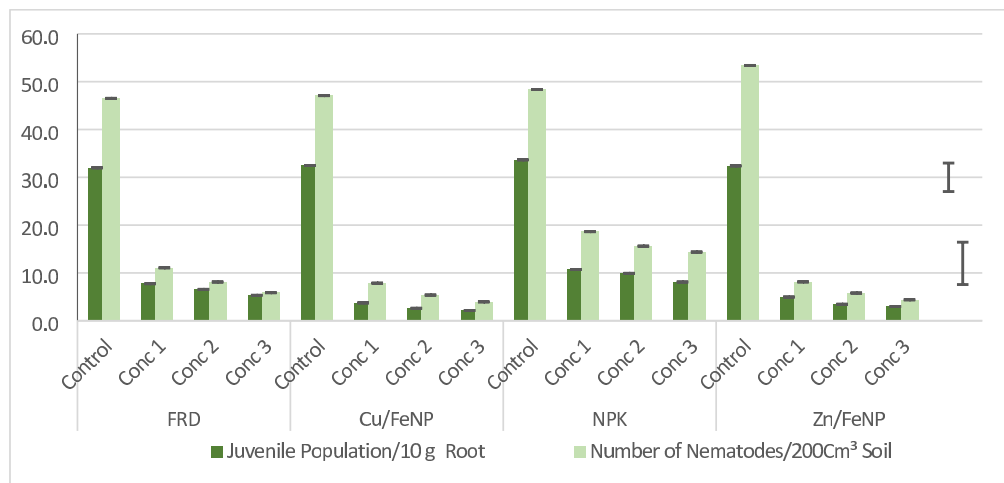


Fig. 8. Effect of treatments and concentration on root and soil nematodes of cabbage plants. Error bar represents standard error of means (SEM) comparing treatments and concentration on root and soil nematodes.

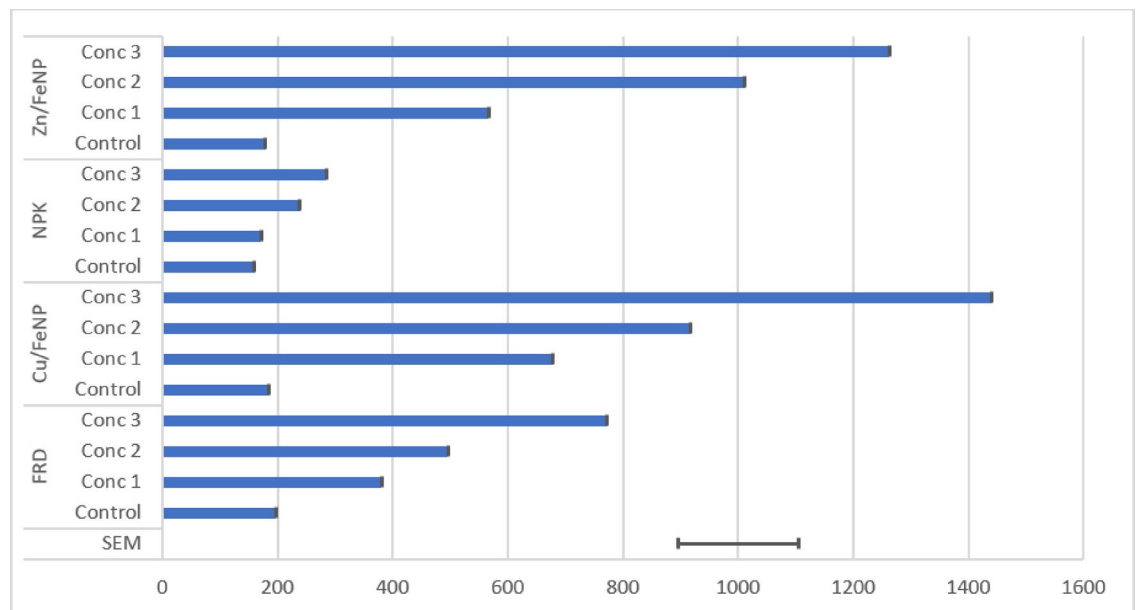


Fig. 9. Effect of treatments and concentration on yield of cabbage plants. Error bars represent standard error of means (SEM) comparing treatments and concentration on yield.

Principal component analysis (PCA)

PC1 (66.78%) and PC2 (27.09%) explain most of the variability. High concentrations of Furadan and Cu/FeNPs were found to correlate with increased inhibition, as they are positioned far from the control (Fig. 15a). Nearly all the variation is accounted for by PC1 (96.17%) and PC2 (2.82%), with different treatments such as Cu/FeNPs and Furadan (in both high and low concentrations), plotted against their effect on juvenile mortality. The clustering around DAT 1 to 6 shows that Cu/FeNPs and Furadan significantly affected the mortality, with Cu/FeNPs 60% having the most significant effect, indicated by its far distance from the centre (Fig. 15b).

From Fig. 15b, PCA 1 contributed 96.17% to the total variation and PCA 2 contributed 2.82%. The result strongly suggests that a dominant single factor was driving the variability. According to the figure, the dominant factor contributing most to PCA1 is the early day mortality responses as recorded at DAT1 and DAT2, thereby suggesting that treatments induced mortality early, i.e. 1–3 days after treatment, are the main drivers of variability as captured by PCA1.

PC1 (82.26%) and PC2 (17.79%) explain nearly the total variance. Variables like head weight of cabbage and nematode population in the soil show strong associations with specific treatments. Nanoparticles impact cabbage head weight, while certain treatments correlate with reduced nematode populations (Fig. 15c). PC1

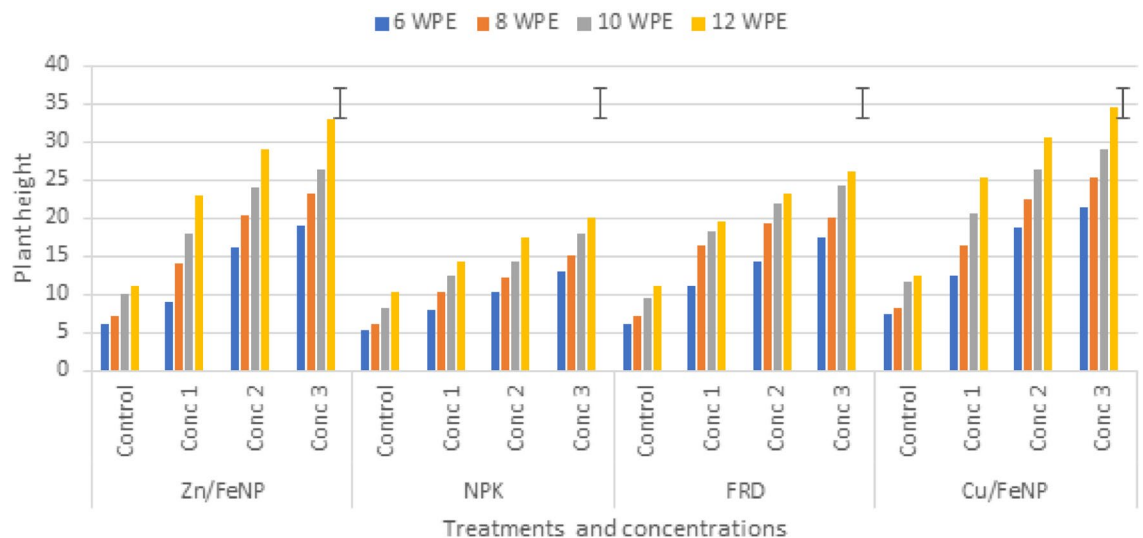


Fig. 10. Effect of treatments and concentration on height of beet root plants. Error bars represent standard error of means (SEM) comparing treatments and concentration on height of plants.

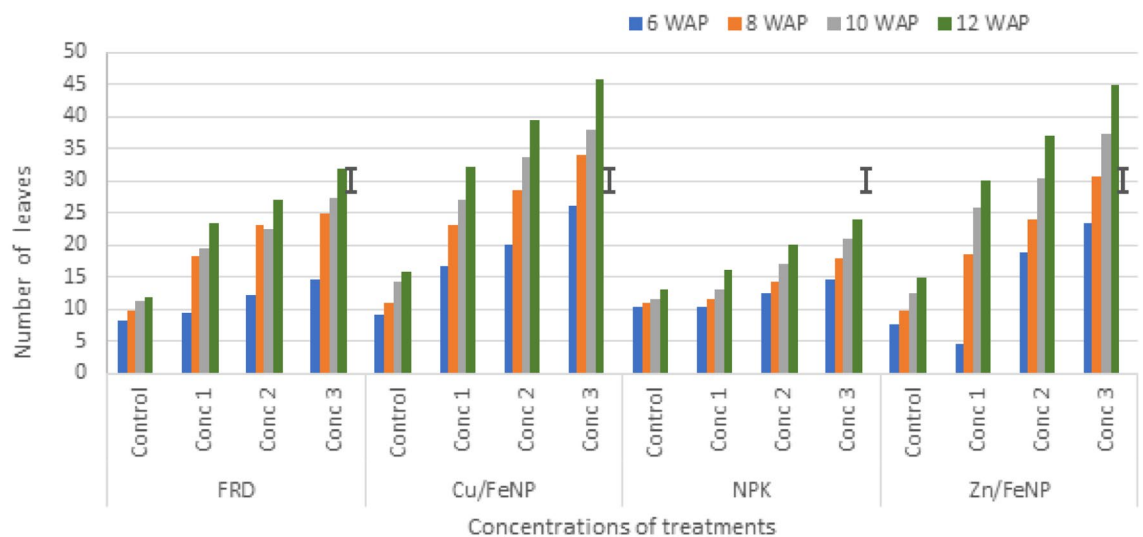


Fig. 11. Effect of treatments and concentration on leaf number of cabbage plants. Error bars represent standard error of means (SEM) comparing treatments and concentration on leaf numbers.

contributed 95.06% and PC2, 4.94% to the total variation. The biplot shows how the treatments impact the gall index, which indicate a measure of nematode infestation. Treatments like Cu/FeNPs and Zn/FeNPs seem to have a stronger effect on reducing egg mass numbers and gall index (Fig. 15d).

PC1 (93.51%) and PC2 (6.75%) explain almost all the variability in cabbage growth. The data suggest that treatments like Zn/FeNPs and Cu/FeNPs enhanced cabbage growth as measured at specific weeks after planting. FRD2 and FRD3 also appear to positively impact cabbage growth during the growth period (Fig. 15e). PC1 (94.3%) and PC2 (5.7%) explain the full variability. This plot shows the correlation between treatments and the gall index in beetroot plants. Some treatments (FRD3, and Cu/FeNPs3) reduce the gall index, indicating lower nematode infestation (Fig. 15f). PC1 (77.50%) and PC2 (22.44%) show the effects of treatments on beetroot yield and nematode populations. Treatments Cu/FeNPs and Zn/FeNPs correlate with improved beetroot yield and reduced nematode populations in both soil and roots (Fig. 15g). PC1 (98.4%) and PC2 (1.07%) almost entirely explain effects of the treatments on beetroot growth over time. Growth data collected over different weeks post emergence (WPE) show positive impacts of various treatments on beetroot growth (Fig. 15h).

Discussion

The production of bimetals is aimed at enhancing potency and characteristics of resulting materials relative to single metal production. A synergistic effect is produced, while the stability and functionalities of both

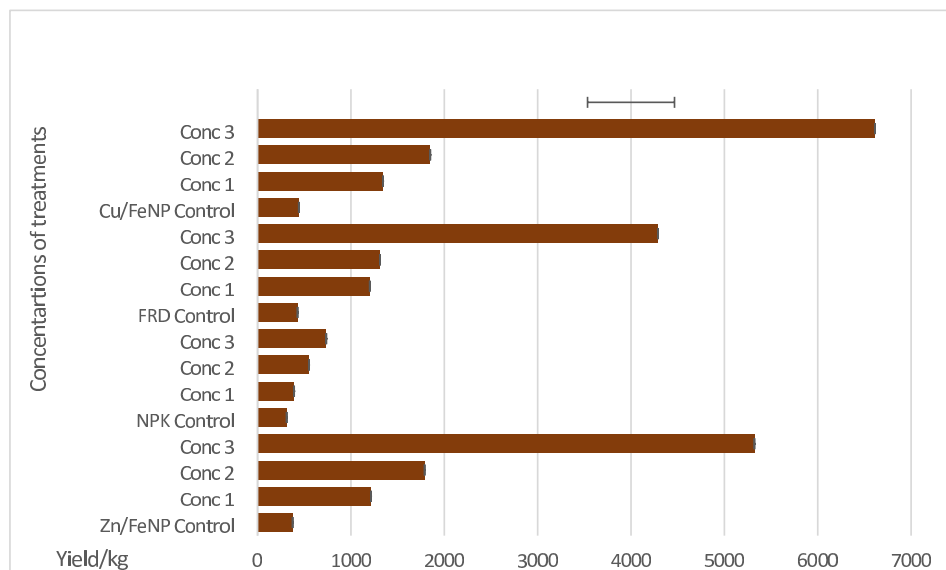


Fig. 12. Effect of treatments and concentration on yield of beet root plants. Error bar represents standard error of mean (SEM) comparing treatments and concentration on yield.

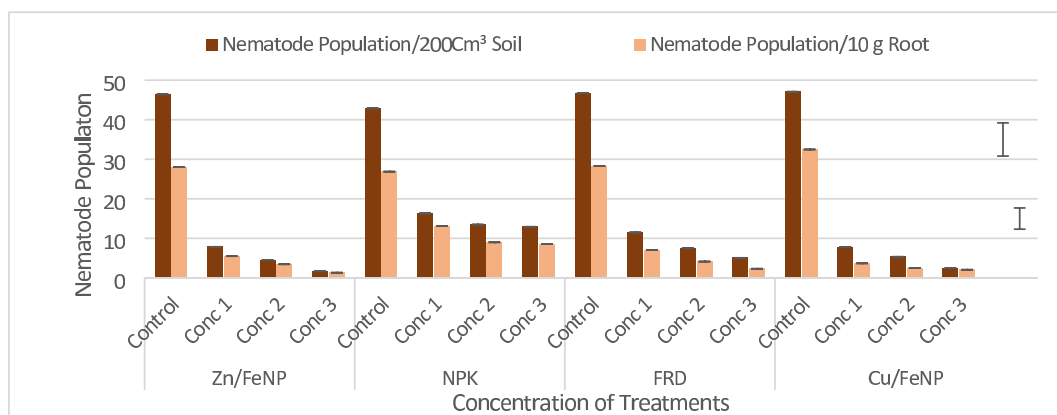


Fig. 13. Effect of treatments and concentration on root and soil nematode of beet root plants. Error bar represents standard error of mean (SEM) comparing treatments and concentration on root and soil nematodes.

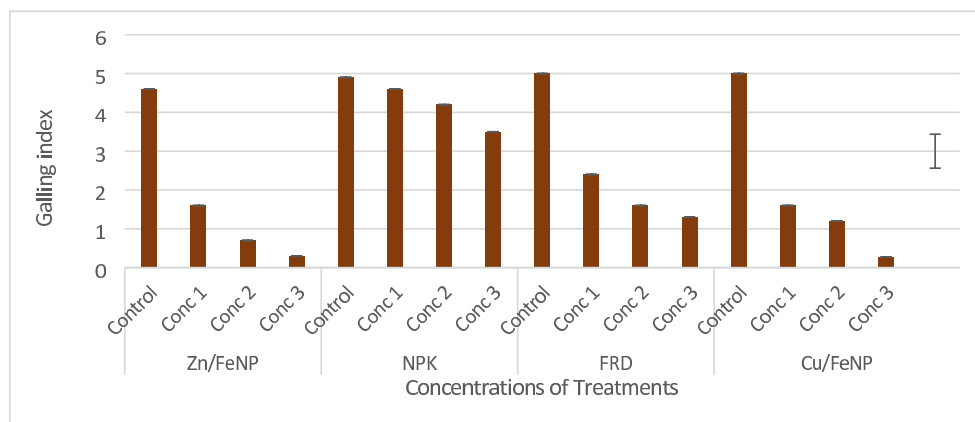


Fig. 14. Effect of treatments and concentration on galling index of beet root plants. Error bar represents standard error of means (SEM) comparing treatments and concentration on galling index.

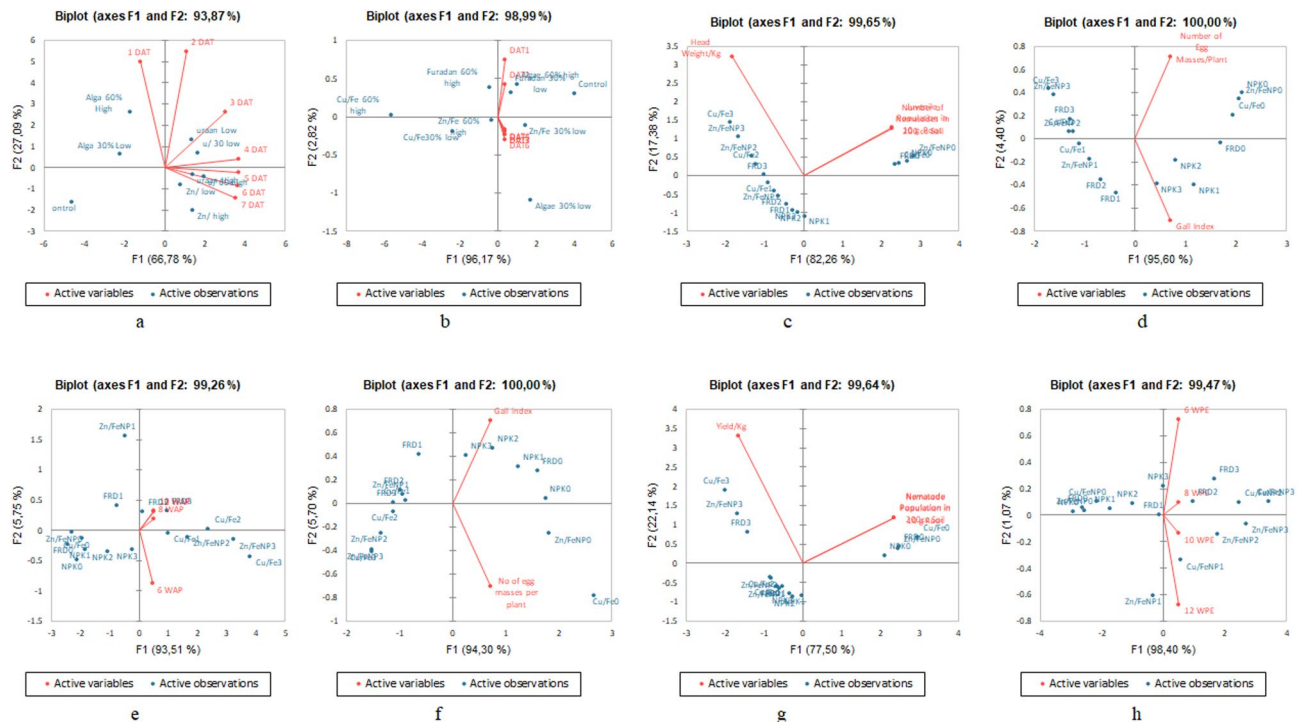


Fig. 15. Principal component analyses of algae and bimetallic nanoparticle treatments and interaction with plant parasitic nematode populations and damage. **(a)** PCA showing hatching inhibition effect of algae, furadan, and bimetallic nanoparticles on *Meloidogyne incognita* eggs. **(b)** PCA of the mortality effect of algae, furadan, and bimetallic nanoparticles on *Meloidogyne incognita* juveniles. **(c)** PCA of the effect of different treatments on the Cabbage head weight and nematode population. **(d)** PCA of the effect of different treatments on the Cabbage egg mass and Gall index. **(e)** PCA of the effect of different treatments on the Cabbage growth (WAP). **(f)** PCA of the effect of different treatments on the Beetroot gall Index. **(g)** PCA of the effect of different treatments on Beetroot nema in soil and roots (Yield, nematode population in 200 cc soil, nematode population in 10 g root). **(h)** PCA of the effect of different treatments on beetroot growth data across varying levels of Zn/FeNP, NPK, FRD and Cu/FeNP at 6WPE, 8WPE, 10WPE and 12 WPE)

metals are combined, thus applications of such materials are broadened⁵¹. Bimetallic nanoparticles have been directed lately to accomplish some sustainable development goals like food security, sustainable water and clean environment owing to their high potency⁵². Bimetal nanoparticles have displayed potent outcomes in water and soil pollution rectification. Efficient antimicrobial action has equally been reported on sundry organisms in the health and food sectors.

Food loss at farm field levels through storage stages is calculated to be above 30%⁵³. In order to minimize such losses, bimetallic NPs are employed in the face of agricultural crop damage challenges. The bimetallics are capable of controlling agricultural pathogens ranging from bacteria, nematodes and fungi. This action is achievable owing to their capability to penetrate pathogen membranes, destroy structures of cells and arrest vital processes of pathogen cells. Hence, activity of pathogen is limited, while oxidative stress is induced. This mechanism is exhibited in fungi, nematodes or bacteria. Bacterial spore and fungal mycelia growth are impacted by cell structural damage, while nematodes become immobile, culminating in cell death of organisms. Thus, NPs and BMNPs are multifaceted materials in agricultural pathogen management. Mode of action of ZnONPs and Ag/ZnONPs on *Alternaria alternata* was evaluated by Daniel et al.⁵⁴ using HRTEM and HRSEM micrographs. They established cell wall modification, decrease in lipase and cellulase action, while NPs penetrated organism cells. Similarly, Tauseef et al.⁵⁵ assessed the effectiveness of CuONPs on *M. incognita* juveniles. Treated J2s were twisted and wrinkled with collapsed cuticle. Application of green route in production of NPs enhances potency of resulting materials, thus hindering proliferation of drug-resistant organisms and forestalling action of sundry pathogens in agriculture^{56,57}. Khan et al.⁵⁸ affirmed potency of medicinal plant mediated NPs in their research on copper oxide nanoparticles. They reported that CuONPs inhibited reproduction and multiplication of nematodes, bacteria and fungi. Hatching of *M. incognita* eggs and juvenile mortality was significantly reduced, while proliferation of mycelium from *Phomopsis vexans* and *Pectobacterium carotovorum* spores was reduced by 85% and 2.5 mm inhibition zone, respectively. Reports by El-Batal et al.⁵⁹ confirm the application of BMNPs as part of the solution to global food insecurity issues. In their report, post-harvest losses of potato tubers to *Alternaria solani* were controlled with Zn/Cu oxide bimetal NPs. The NPs acted as protective coatings for potato tubers in storage. Similarly, strawberry root rot fungal pathogens were inhibited by bimetallic Ag/Cu NPs⁶⁰. The researchers reported that foliar application of BMNPs at 200 ppm significantly minimized strawberry grey mould and root rot. Likewise, several fungal strains (*Sclerotinia sclerotiorum*, *Fusarium venenatum*, *Fusarium*

equiseti and *Alternaria* sp.) pathogenic to tomato plants were controlled by Ag/Fe bimetal NPs (10 mg/mL) mediated with *Adansonia digitata* fruit shell extract⁶¹.

In the current study, copper/iron and zinc/iron NPs displayed efficiency in the control of *M. incognita* populations based on female count and galls on beetroot and cabbage plant roots after harvest. This observation is corroborated by the findings of Gkanatsiou et al. [62], who found Cu/FeO NP bimetal to be highly effective on *M. incognita* and *M. javanica*. Biological cycle was arrested and paralysis was induced at 73 µg/mL after 24 h of treatment. In pot and laboratory experiments, respectively. Copper, iron and zinc NPs synthesized individually with *Tridax procumbens* and *Borreria verticillata* have been established to be nematocidal. Significant reduction in egg mass, J² in roots and gall index were noted with an appreciable yield in cabbage and cowpea correspondingly^{21,63}. Copper and iron associated with the class of trace elements for crop use, are classified as requisite to crop development. They both function at cellular level and support crops' photosynthetic and biological processes. Deficiencies have been reported to result in crop structural failures⁶⁴. Iron NPs link with add-on forms in soil. NPs transform into ionic bio-accessible mode for onward movement into tissues of plants. Irons are assessed by plants in the ionic states such as Fe²⁺ and Fe³⁺, while Fe²⁺ is known to stimulate development of crops. It is conjectured that from Cu/Fe NPs, iron was transfigured to ionic state and made available to crops in a blend with soil micronutrients, hence the potency demonstrated on *M. incognita* populations in this study⁶⁵. Uptake of copper by plants could be influenced by factors like soil constituents and properties. Copper is primarily linked through absorption to organic and inorganic matter. Components of soil make binding sites available to copper ions, which equally exhibit significant attraction for such binding sites. Absorption of copper could equally take place on surfaces like manganese, iron, or clay; it may be present in silicate mineral lattices or solubilized with phosphates or carbonates⁶⁶.

Conclusion

Pests, pathogens and climate variability are primary contributors to food insecurity. Bimetal NPs could be employed to manage pests, pathogens and mitigate damages and difficulties from variability in climate, for environmentally conscious crop development. Bimetal production is a platform to upscale metallic NPs in connection with accuracy, dependability and harmlessness. Phyto constituent variation and concentration of plant extracts in bimetal productions are significant components that are likely to impact potency of final product. Application of plant extracts in the production of bimetal NPs supports strength of such NPs and affords low toxicity attributes on the NPs. The remarkable activity of BMNPs was observed in *in vitro* studies, where contact with organisms confirmed effectiveness of Cu/FeNPs and Zn/FeNPs at 60% concentration. Correspondingly, 100 ppm of Cu/FeNPs and Zn/FeNPs afforded protection against *M. incognita* parasitising beetroot and cabbage plants under field condition. Potency of materials may rest on climatic fluctuations, location, pH and components of soil. Thus, bimetal NPs can be tailored to avenues of action focused context to accomplish SDG goals premised on secured food, water, and clean environment. Future research on assessment of BMNPs on other significant plant parasitic nematodes parasitising cereal and legumes is suggested, while application of bimetal NPs concerning multiplex aspects of supportable progress is proposed.

Data availability

The datasets used for the current study are available from the corresponding author on reasonable request.

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Author contributions

FOA....Conceptualization; supply of materials; design; methodology; benchwork; data collection; data analysis; prepared Figs. 3 and 7; original draft and review. AHL....Supply of materials, design; benchwork; data collection; prepared Figs. 1, 2 and 3; original draft AVO....Funding source, original draft, review and editing ATA....Supply of materials; original draft HSM....Funding source OBA....Original draft and review. AOCC....Supply of materials; prepared Figs. 4, 5 and 6; original draft; editing.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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