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ORIGINAL PAPER

Evaluation of feather hydrolysate-mediated silver nanoparticles as biofertilizers for the enhancement of vegetative growth and nutraceutical properties of vegetables

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Abstract

Agricultural production currently depends largely on the use of synthetic fertilizers to boost the production of staple and healthy foods. However, their excessive and inappropriate use had been found expensive and detrimental to the ecosystem. Thus, development of alternative bio-based fertilizers in the form of nanomaterials to improve the yield and nutraceutical properties of crops in a sustainable manner is encouraged. This study therefore reports the biosynthesis of silver nanoparticles (FH-AgNPs) using feather hydrolysates (FH) obtained after chicken feather degradation by keratinolytic *Bacillus safensis* LAU 13 and *Aquamicrobium defluvii* FH 20. Phytostimulatory effects of the biogenic AgNPs on *Corchorus olitorius*, *Amaranthus caudatus* and *Celosea argentea* cultivated in soil treated with 50–150 µg/ml FH-AgNPs were investigated compared to NPK fertilizer (15–15-15) and water as positive and negative controls, respectively. Vegetables grown with 150 µg/ml of both FH-AgNPs demonstrated 1–1.58-fold improvement in seed germination, shoot height, root length, leaf size, chlorophyll contents and other growth parameters compared to their controls. Hydrogen peroxide and DPPH radicals scavenging activities of the FH-AgNPs-fertilized vegetables were over 1.1-fold better than their respective control plants. FH-AgNPs treatment enriched the total phenolic, flavonoids, and proanthocyanidin compounds in the vegetables by more than 1.05-fold. The particles positively influenced the catalase activity of the vegetables and also inhibited lipid peroxidation in precision-cut liver slices by 1.05–1.21-fold over the untreated plants. The FH-AgNPs demonstrated inhibitory activities (60.33–88.20%) against phytopathogenic *Aspergillus niger*, *Aspergillus flavus* and *Fusarium solani*. Application of the biogenic FH-AgNPs performed considerably better than the NPK fertilizer virtually in most cases, aside their usefulness as nanopesticides. Thus, results obtained in this study indicate that the FH-mediated AgNPs have potential application as better substitute to conventional inorganic fertilizer to promote sustainable agricultural food production in an eco-friendly manner.

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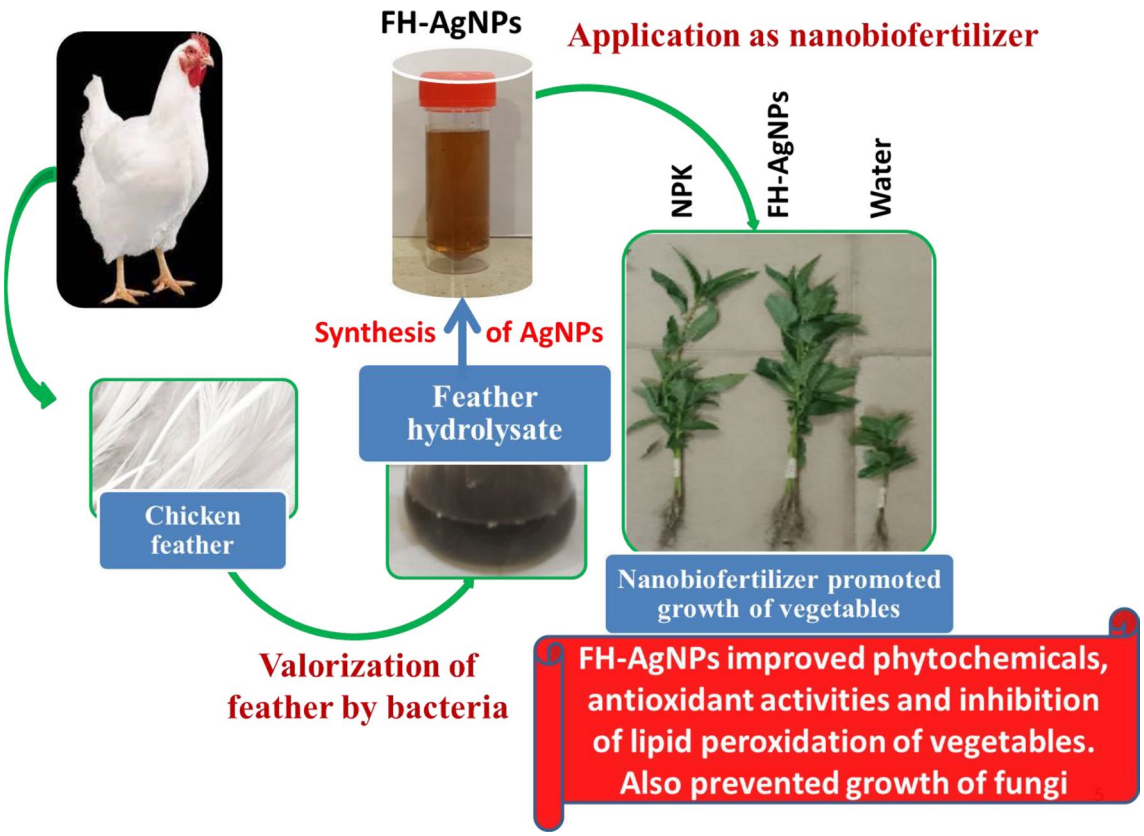
Graphical abstract



Keywords Feather hydrolysates · Lipid peroxidation · Phytopathogens · Phytostimulatory effect · Silver nanoparticles · Vegetables

Introduction

Vegetables are very important part of balanced diet as they are good sources of nutrients and nutraceutical compounds. They are tagged as a protective food since they are rich in carbohydrates, proteins, vitamins and minerals. They render tremendous health protection owing to their richness in phytochemical compounds like terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, polyphenols, flavonoids, isoflavonoids and anthocyanidins [1, 2]. Regular intake of fruits and vegetables lower the risk of chronic diseases such as cardiovascular diseases, cancer, diabetes as well as some digestive disorders like flatulence, constipation and diarrhea. They are so important in the daily diet such that World Health Organization (WHO)-recommended consumption of 400 g/d of fruits and vegetables for better health protection [3, 4]. Adequate consumption of fruits and vegetables lowers the severity and recovery from some infectious diseases [5].

Vegetables are globally cultivated and make up a major portion of the diet of humans in many parts of the world [2]. They are essential for maintenance of good health; their productions are veritable sources of income generation and have potential to increase food security and generate employment [6]. The consumption of vegetables and fruits is so pertinent that the General Assembly of the United Nations [7] declared year 2021 as the international year of fruits and vegetables (IYFV). The growth and performance yield of vegetables are controlled by soil conditions such as availability of nutrients, pH, soil texture and presence of various beneficial microbial population [8]. Conventionally, synthetic fertilizers are often used to boost the growth and performance yield of vegetables; however, excessive and inappropriate use of chemical fertilizers had been found unsustainable and harmful to the ecosystem [9]. Thus, strategies like nanoagriculture and organic farming through the application of feather hydrolysates have been deployed to improve the yield and nutraceutical properties of vegetables in a sustainable manner [10–15].

Nanoparticles as the building block of nanotechnology influence various biological activities in plants such as plant growth, yield, health and other physiological processes [16]. Physicochemical properties of nanoparticles most

importantly large surface to volume ratio and chemical compositions make them very suitable for agricultural practices [17]. Biogenic nanoparticles are most preferred for various biological applications unlike chemically synthesized particles owing to their simplicity in production, less toxicity, eco-friendliness and biocompatibility nature [18, 19]. Phytostimulatory effects of metallic nanoparticles have been well documented and are ascribed to their abilities to penetrate, translocate, facilitate electron exchange and develop favorable interactions with vegetable plants which enhance photosynthetic process, water utilization, nitrogen metabolism, seed germination, cell division and elongation to eventually increase their growth, promote their physiological activities and diseases control [10, 20]. They are more efficient in supplying nutrients to plants as they maintain gradual and continuous release of nutrient to plants [21].

Nanotechnology have made remarkable impact in vegetable production as it improved seed germination, seedling growth, abiotic and biotic stresses tolerance, yield and quality enhancement [22]. AgNPs are capable of inducing enlargement of root pores to facilitate the uptake and translocation of nutrients. They have stimulated plant growth, enhanced seed germination, improved antioxidant activity and regulated soil phytopathogens [11, 23]. Phytopathogens have detrimental effect on plants as they mediate diseases that pose significant loss of yield and quality. Conventionally, control measures such as the use of fungicides have been adopted but with many limitations such as soil toxicity, high cost and incomplete eradication of pathogens. However, application of green nanoparticles had emerged as one of the better alternatives for the effective management of phytopathogens in a sustainable manner. AgNPs possess wide spectrum of antimicrobial properties and have gained importance as potent antiphytopathogenic agents that could kill a vast varieties of plant pathogens [24].

Metallic nanoparticles enhanced biosynthesis of phytochemicals that protects plants against environmental stress and infections. They boosted chlorophyll contents, antioxidant activities and enriched nutritional components of vegetables [25]. Roy and Anantharaman [26] reported plant growth-promoting effect of AgNPs synthesized from aqueous extract of brown seaweed *Sargassum ilicifolium*. The particles enhanced the seed germination of *Abelmoschus esculentus* and *Raphanus sativus* by 40%. Recently, Azeez et al. [11] reported improvement in the antioxidant and ferric-reducing activities of *Corchorus olitorius* cultivated on AgNPs-treated soil.. There exist paucity of informations on application of biogenic metallic nanoparticles to improve vegetative and nutraceutical quality of vegetables; thus, there is need to expand the scope of applications of biogenic nanoparticles as nanofertilizers in the cultivation of more vegetables.

In this study for the first time, feather hydrolysates-mediated AgNPs were evaluated on the growth, phytochemicals composition, antioxidant and hepatoprotective properties of *Corchorus olitorius, Amaranthus caudatus* and *Celosia argentea* cultivated under pot conditions.. These vegetables are widely consumed in Nigeria and have well-documented biological properties that promote their consumption for nutritional and health benefits [27–30]. Earlier, we have shown that bacterial feather hydrolysates promoted the growth of the studied vegetables in soil-less Petri dish and pot experiment [31]. The feather hydrolysates were produced via microbial valorization of chicken feather by *Bacillus safensis* and *Aquamicrobium defluvii,* and exploited for the biosynthesis of AgNPs, which were used as nanofertilizers to grow the vegetables.

Materials and methods

Collection of feather and planting materials

Chicken feathers were obtained from local farms in Ogbomoso and screened to remove extraneous materials. The feathers were washed and dried at 75 °C, after which milling was done to grind the feathers into powder [31]. The powdered feather was stored in air-tight container until further use. The vegetable seeds, *Corchorus olitorius* var. *corete potagere*, *Celosia argentea* var. *cristata,* and *Amaranthus caudatus* var. *hemera*, were procured from Farm Help Agrostores, Moniya, Ibadan, Oyo State, Nigeria.

Isolation of keratinolytic bacteria

The keratinolytic *Bacillus safensis* LAU 13 that was isolated from a feather dump site [32] was obtained from the culture collection of Laboratory of Industrial.

Microbiology and Nanobiotechnology, LAUTECH, Ogbomoso. Novel keratinolytic bacterial strain was isolated from soil sample collected from the same site where the feather wastes were obtained. Soil sample of 1 g was dispersed in 9 ml of sterile distilled water, and 0.5 ml aliquot was inoculated into the minimal medium compounded using feather powder and mineral salts for the selective growth of bacterial isolates using pour plate method; the plates were held at 37 °C for 3 days. Thereafter, distinct colonies observed through morphological features were sub-cultured on yeast extract agar plates to obtain pure cultures. The obtained pure cultures were preserved on agar slants of yeast extract agar and minimal selective medium for further use [32]. The isolate was thereafter characterized and identified as *Aquamicrobium defluvii* FW 20 (KU163265.1) using 16S rRNA analysis.

Bioconversion of chicken feather wastes into feather hydrolysate

Each of the keratinolytic bacterial strains, *Bacillus safensis* LAU 13 (KJ461434) and *Aquamicrobium defluvii* FW 20 (KU163265.1) that were earlier reported [31, 32], was used for the inoculum development by inoculating into a medium containing 1% keratin powder, 0.2% yeast extract, pH 7.5 and incubated at 37 °C, 100 rpm for 24 h. Feather hydrolysis was carried out by inoculating 1 ml of inoculum into 19 ml of feather-based medium in 100-ml flasks [32]. Cultures were incubated at 37 °C, 100 rpm for up to 7 days, thereafter, whole content of each flask was collected, centrifuged at 5000 rpm for 20 min, and the supernatant was heated at 60 °C for 30 min to terminate the bacterial cultures. The heated supernatants served as feather hydrolysates that were used without further purification.

Biosynthesis of silver nanoparticles using bacterial feather hydrolysates

Biosynthesis of FH-AgNPs was carried out using feather hydrolysates obtained after the hydrolysis of chicken feather by *B. safensis* LAU 13 and *A. defluvii* FH 20 as previously reported by Lateef et al. [33]. Feather hydrolysate of 1 ml was added into the reaction vessel containing 40 ml of 1 mM silver nitrate ($AgNO_3$) solution for the reduction of silver ion. The reaction was held under sunlight without agitation for 2 h. Reaction was visually monitored to observe color change, and measurement of absorbance spectrum of the reaction mixture was done using UV–Visible spectrophotometer (Genesys 10 UV Thermoelectron Corporation, UK) at 300–800 nm.

Investigation of biomolecules responsible for the formation of FH-AgNPs was carried using Fourier transform infrared (FTIR) spectroscopy (BUCK M530 Spectrophotometer, Buck, USA) [34]. Solid residue of the AgNPs obtained after centrifugation was dried at room temperature and mixed with potassium bromide (KBr) pellets for the FTIR measurements. Determination of sizes and morphology of the particles were done using transmission electron microscopy (TEM) by applying a drop of colloidal FH-AgNPs on a 200 mesh hexagonal copper grid. This was coated with 0.3% formvar dissolved in chloroform and then air-dried before examination under transmission electron microscope JEM-1400 (JEOL, USA) operating at 200 kV.

Evaluation of plant growth-promoting effect of biosynthesized silver nanoparticles on vegetables

The modified method described by Azeez et al. [11] was adopted in carrying out this investigation. The experiment was conducted at the Botanical Gardens, Department of Pure and Applied Biology, LAUTECH Ogbomoso, Oyo State, Nigeria (8.1650° N, 4.2763° E), between October and December, 2021. Soil sample (500 g) from the study site was collected inside each 3-L capacity dark polythene bag for the evaluation of plant growth-promoting effects of the biosynthesized FH-AgNPs on the three leafy vegetables *Corchorus olitorius*, *Celosia argentea* and *Amaranthus caudatus* by considering different concentrations of FH-AgNPs (50, 100 and 150 µg/ml). Fifteen (15) seeds were planted per pot at an average depth of 1–2 cm and flooded immediately after planting with 100 ml of each FH-AgNPs concentrations. The positive control pot was treated with 2.5 g of NPK fertilizer (15:15:15) 5 days ahead of planting to allow the nutrients in the fertilizer to be readily available for the seedling growth, while the negative control pot was devoid of fertilizer and FH-AgNPs. Each of the positive and negative control pots was flooded with 100 ml of tap water immediately after planting. Subsequently, the test experiment pots were irrigated with 100 ml of their corresponding FH-AgNPs concentrations two weeks after planting, while water was used for control pots. Pots were perforated to allow excess water drain out, and they were arranged under a net. The experiment was a randomized complete block design with four replicates. Seedlings were later thinned two weeks after planting to allow sunlight penetration, adequate air circulation and to reduce competition for nutrients. Five stands were thereafter maintained per pot, and the vegetables were grown for five weeks before harvesting.

Determination of plant growth characteristics

Growth parameters of the harvested plants including shoot length, root length, vigor index, germination percentage and others were determined. The shoot height and root length were measured using metric rule. The shoot fresh weight was taken, and dry weight was determined after drying at 60 °C to a constant weight. Germination rate index was determined according to the method described by Esechi [35]. The vigor index and germination percentage were calculated as follows:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Number of seeds planted}} \times 100$$

Vigor index = (mean of shoot height + mean of root length) × germination percentage.

Germination rate index (GRI) $= \frac{G1}{1} + \frac{G2}{2} + \frac{G3}{3} + \ldots \frac{Gx}{x}$, where G1 is the germination percentage after one day of sowing; G2 is the germination percentage after two days of sowing, G3 is the germination percentage after 3 days of sowing, and Gx is the germination percentage after x days of sowing.

The leaf area was determined using Montgomery equation (ME) according to He et al. [36].

Montgomery equation (ME).

$$\text{Leaf area} = \text{leaf length (m)} \times \text{leaf width (m)} \times 0.68(\text{Montgomery parameter})$$

The chlorophyll content was determined as described by Palta [37] with little modifications. Fresh leaf of each vegetable plant (30 mg) was submerged in a test tube containing 7 ml of 80% acetone and incubated in the dark for 72 h. Thereafter, absorbance readings at 645 and 663 nm were taken and the total chlorophyll content was determined using the following expression:

$$C = \frac{20.2A645 + 8.02A663 \times Y}{1000 \times n}$$

C is the total chlorophyll contents (mg/g FW), A_{645} and A_{663} are the absorbance of the extract at 645 and 663 nm, *Y* is volume of extract, and *n* is leaf weight.

Determination of antioxidant activities of the vegetables

The method of Brand-Williams et al. [38] was adopted for DPPH radical scavenging assay by reacting 1 ml of graded concentrations of methanolic extract of each vegetable plant (4 mg/ml) with 4.0 ml methanolic solution of 0.1 mM DPPH. The mixture was shaken and allowed to stand in a dark box for 30 min at room temperature (30 ± 2 °C). One milliliter absolute methanol mixed with 4.0 ml of 0.1 mM methanolic DPPH was also prepared and used as blank. Absorbance of the resulting solution was measured at 517 nm on a UV–Vis spectrophotometer. Percentage DPPH radical scavenging activity (% DRSA) was calculated by the following equation:

$$\%\ \text{DRSA} = \frac{A0 - A1}{A0} \times 100$$

where Ao is the absorbance of the control, and A_1 is the absorbance of the test extract.

Hydrogen peroxide scavenging activity of the vegetables extracts was determined according to methods described by Gülçin et al. [39]. Extracts (50–800 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM), incubated for 10 min, and absorbance of the resulting solution was read at 230 nm. Hydrogen peroxide solution was used as control, while distilled water was used as blank. Percentage of hydrogen peroxide scavenged by the extracts and a standard compound was calculated as follows:

$$\%\ H_2O_2\text{scavenged} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Determination of Lipid Peroxidation Inhibition and Catalase Activities of the Vegetables.

The lipid peroxidation inhibition (LPI) was determined according to the method described by Liu and Ng [40]. Excised liver of a freshly sacrificed albino rat was homogenized in phosphate buffer (10%, w/v) and then centrifuged to obtain homogenate. Liver homogenate of 0.5 ml was added with 0.1 mL of each vegetable extract (10 µg/ml) then with $FeSO_4$ (50 µl, 10 mM) to make final volume of 1 ml. The reaction mixture was incubated at 37 °C for 20 min. Thereafter, TCA (1 ml, 28%) and 1.5 ml of (1%) TBA were added and heated at 100 °C for 15 min. After cooling, the absorbance was taken at 532 nm. The control consisted of same compositions without the vegetable extract.

Percentage inhibition of lipid peroxidation (% LPI) was calculated using the following equation:

$$\%\ \text{LPI} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the control, and A_{sample} is the absorbance of the test sample.

Catalase activity assay was carried out following the method described by Cohen et al. [41] with modifications. Briefly, the mixture of vegetable extract (75 µl) and liver homogenate (125 µl) inside an ice cold tube was added with phosphate buffer (1.0 ml, pH 7.4) and hydrogen peroxide (0.8 ml, 30 mM). Tube contents were then mixed thoroughly by inversion. Reaction was stopped after 3 min with the addition of 1 ml, 6 M H_2SO_4. Potassium permanganate (3 ml, 0.01 M) was then added and absorbance reading at 480 nm within 30–60 s was taken, and catalase activity was determined thus:

$$\text{Catalase enzyme activity} = \frac{\text{Absorbance/min} \times y \times 1000}{M \times V \times W}$$

V is total volume of the reaction mixture; *M* is molar extinction coefficient which is 40.0, *W* is weight of tissue; and y is volume of sample used.

Phytochemical analysis of the vegetables

Total phenol content was estimated using a modified method described by McDonald et al. [42]. Briefly, 0.5 ml of each plant extract (1:10 g/ml) was mixed with 5 ml of Folin–Ciocalteu reagent (1:10,diluted with distilled water) and 4 ml of aqueous 1 M Na_2CO_3. The mixture was heated at 40 °C for 30 min, and the absorbance was read at 765 nm. Total phenol content was expressed as mg/g of gallic acid equivalent (GAE) using expression:

$y = 0.0086x + 0.0105$, x is the absorbance and y is the gallic acid equivalent (mg/g).

Total flavonoid content was determined according to the procedure of Zhishen et al. [43] with little modifications. Each vegetable extract (0.1 ml) was added to 0.3 ml distilled water followed by addition of 5% $NaNO_2$ (0.03 ml). After 5 min of reaction under 25 °C, $AlCl_3$ (0.03 ml, 10%) was added. After another 5 min, the reaction mixture was added with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. Catechin was used as a standard for the calibration curve. The result was expressed in terms of catechin equivalent (CAE), using expression:

$y = 0.0135x + 0.0085$, x is the absorbance and y is the catechin equivalent (mg/g).

Determination of proanthocyanidin was carried out as described by Sun et al. [44]. The vegetable extract (0.5 ml, 0.1 mg/ml) was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid, the mixture was allowed to stand for 15 min, and thereafter, absorbance was taken at 500 nm. Total content of proanthocyanidin was expressed in terms of catechin equivalent (CAE) using expression:

$y = 0.0135x + 0.0085$, x is the absorbance and y is the catechin equivalent (mg/g).

Antifungal activities of biosynthesized FH-AgNPs against phytopathogens

Evaluation of antifungal activity of AgNPs biosynthesized from each of FHs obtained aftermath of feather degradation by *B. safensis* and *A. defluvii* was carried out as described by Akinola et al. [45]. Silver nanoparticles (150 µg/ml) were added to molten potato dextrose agar (PDA) (1:10, v/v). Thereafter, sterile cork borer was used to cut 6-mm-diameter mycelial plug from 48-h-old cultures of *Fusarium solani*, *Aspergillus flavus* and *Aspergillus niger*. The plugs were placed at the center of PDA plates. The control experiment consisted of fungal plug inoculated on PDA plate devoid of FH-AgNPs incorporation. The plates were incubated under ambient conditions (30 ± 2 °C) for 72 h; afterwards, diameters of fungal growth in all plates were measured and percentage of fungal growth inhibition was determined as follows:

$$\%\ \text{of fungal growth inhibition} \frac{D_{\text{control}} - D_{\text{test}}}{D_{\text{control}}} \times 100$$

D is the diameter of fungal growth on the PDA plate.

Statistical analysis

Data obtained in this study were analyzed using SPSS statistical package version 20. Values were expressed as means ± SEM and evaluated for significant difference through ANOVA ($p < 0.05$) and Duncan's multiple range tests.

Results and discussion

Biosynthesis of FH-AgNPs

Feather hydrolysates obtained upon degradation of chicken feather by keratinolytic *B. safensis* LAU 13 and *A. defluvii* FH 20 [31] facilitated the biosynthesis of brown colloidal AgNPs (Fig. 1) that exhibited maximum absorbance at the wavelength of 410 and 416 nm, respectively (Fig. 2). The mixture of FHs and $AgNO_3$ solution immediately produced light yellow coloration which later developed gradually to stable brown after 30 min of reaction. The color change was due to excitation of surface plasmon resonance induced by reduction of silver ion by biomolecules present in the FHs [33]. Biosynthesis of nanoparticles is a bottom-up approach where the nanoparticles are synthesized through the reduction of metallic ions to zero-valent forms by the organic moieties derived from plants, animals and microorganisms [32]. Earlier, we showed that the degradation of feathers by the two bacterial isolates led to the release of amino acids and soluble proteins which can reduce Ag^+ to Ag^0 [46] followed by the nucleation of the reduced metal atoms to form AgNPs as previously demonstrated [33]. The maximum absorbance peaks demonstrated by the particles correspond with AgNPs absorbance characteristics that were previously reported [34, 47]. FTIR spectrum of the biogenic *B. safensis* FH-AgNPs showed prominent peaks at 1640 and 3257 cm^{-1}, while 1636 and 3280 cm^{-1} were recorded for FH-AgNPs synthesized using *A. defluvii* feather hydrolysate (Fig. 3). The bands 3257 and 3280 cm^{-1} refer to the bonding vibration of amines (N–H group) of proteins, while 1640 and 1636 cm^{-1} refer to C = C stretch of alkenes or C = O stretch of amides, indicating that protein molecules in the FHs obtained after the microbial degradation of chicken feather wastes were responsible for the capping and stabilization of the biosynthesized FH-AgNPs. There was also the presence of alkynes

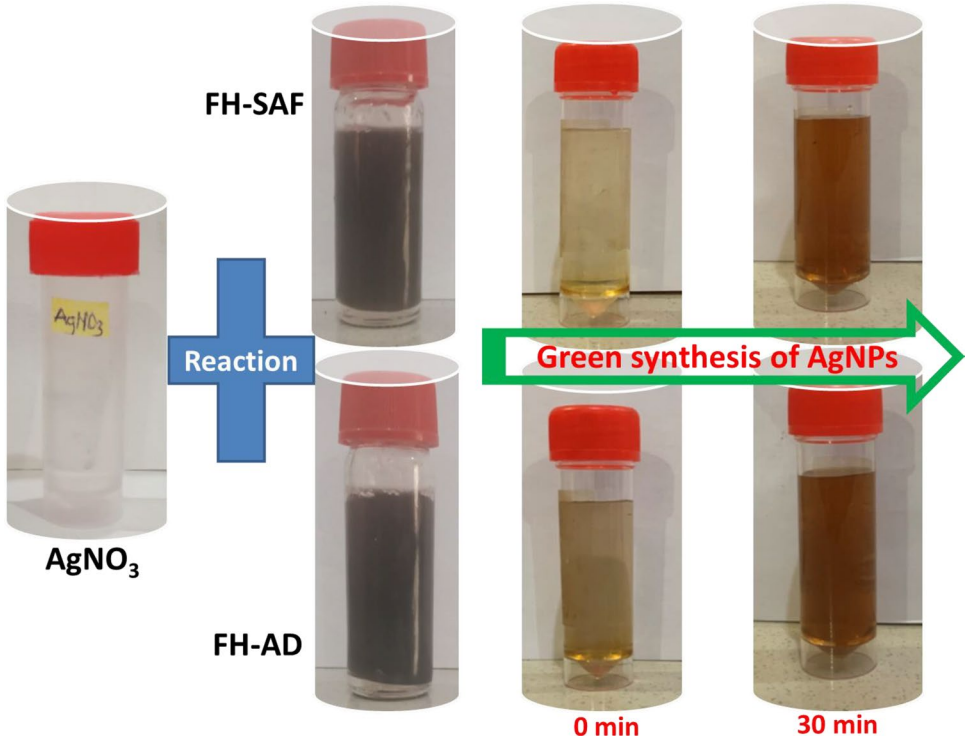


Fig. 1 Biosynthesis of AgNPs using feather hydrolysates produced by *B. safensis* (SAF) and *A. defluvii* (AD) within 30 min of reaction under solar irradiation

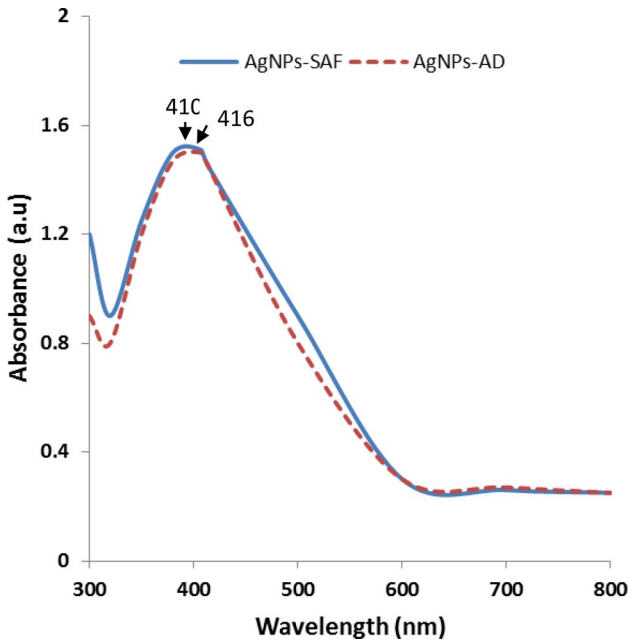


Fig. 2 UV–Vis absorption spectra of AgNPs synthesized from feather hydrolysates produced by *B. safensis* (AgNPs-SAF) and *A. defluvii* (AgNPs-AD)

(C-H) in the colloidal solutions. Earlier, Lateef et al. [33] showed that AgNPs produced using microbial keratinase had prominent peaks at 3410, 1664 and 600 cm^{-1}. The morphology and size of the biosynthesized AgNPs from *B. safensis* and *A. defluvii* feather hydrolysates as appeared in TEM images revealed that they are mainly spherical with average sizes of 42.01 ± 20.9 nm and 11.52 ± 6.37 nm, respectively (Fig. 4a). Also, the selected area electron diffraction (SAED) images showed ring-like patterns indicating the formation of crystalline FH-AgNPs (Fig. 4b), while the EDX showed the presence of Ag (Fig. 4c) in addition to the presence of other elements that may be derived from keratin hydrolysis by the bacteria. The characteristic features of the biosynthesized FH-AgNPs as obtained in this study correspond with the results obtained in the previous and related findings [47–49]. In a recent study, Ullah et al. [50] reported the synthesis of spherical-shaped AgNPs using chicken feather extract with size range of 3–13 nm and SPR around 400 nm.

Effect of FH-AgNPs on the growth and photosynthetic parameters of vegetables

The biosynthesized AgNPs impacted positively on the germination and growth of *Corchorus olitorius*, *Amaranthus caudatus* and *Celosia argentea* seeds after 5 weeks of cultivation (Fig. 5). The *C. olitorius*, *A. caudatus* and *C. argentea* seeds cultivated with the optimal concentration of FH-AgNPs biosynthesized from *B. safensis* FH displayed higher germination rate index (GRI), shoot height (SH), root length (RL), shoot fresh weight (SFW), shoot dry weight (SDW), leaf number (LN), leaf area (LA) and chlorophyll content compared to their respective controls (Table 1). For instance, *C. olitorius* treated with 150 µg/ml FH-AgNPs

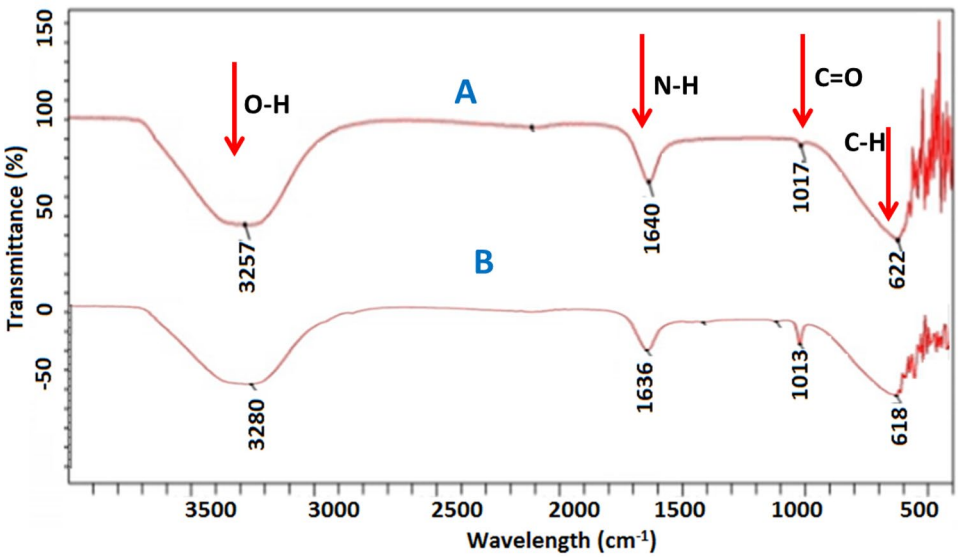


Fig. 3 FTIR spectra of the biosynthesized AgNPs using feather hydrolysate of *B. safensis* LAU 13 (**A**) and *A. defluvii* FH 20 (**B**)

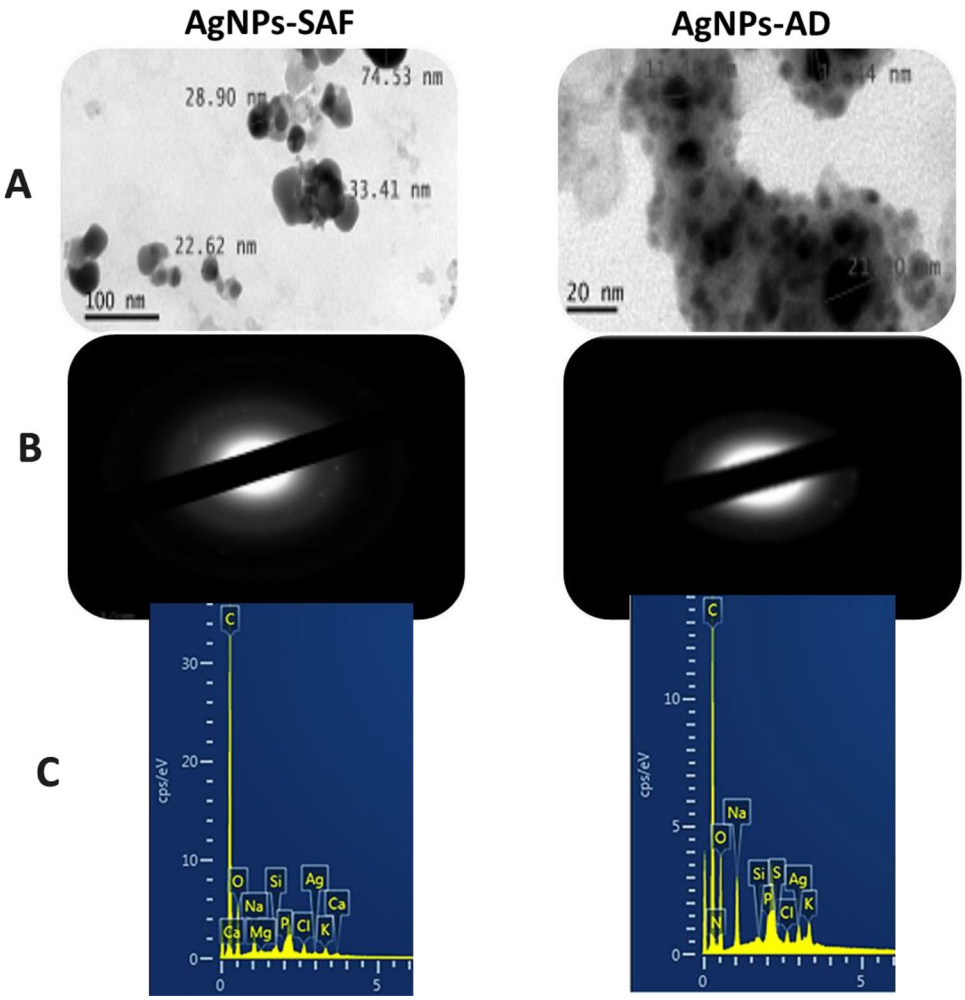


Fig. 4 TEM image (**A**), SAED pattern (**B**) and EDX (**C**) of silver nanoparticles synthesized using feather hydrolysates of *B. safensis* LAU 13 and *Aquamicrobium defluvii* FH 20

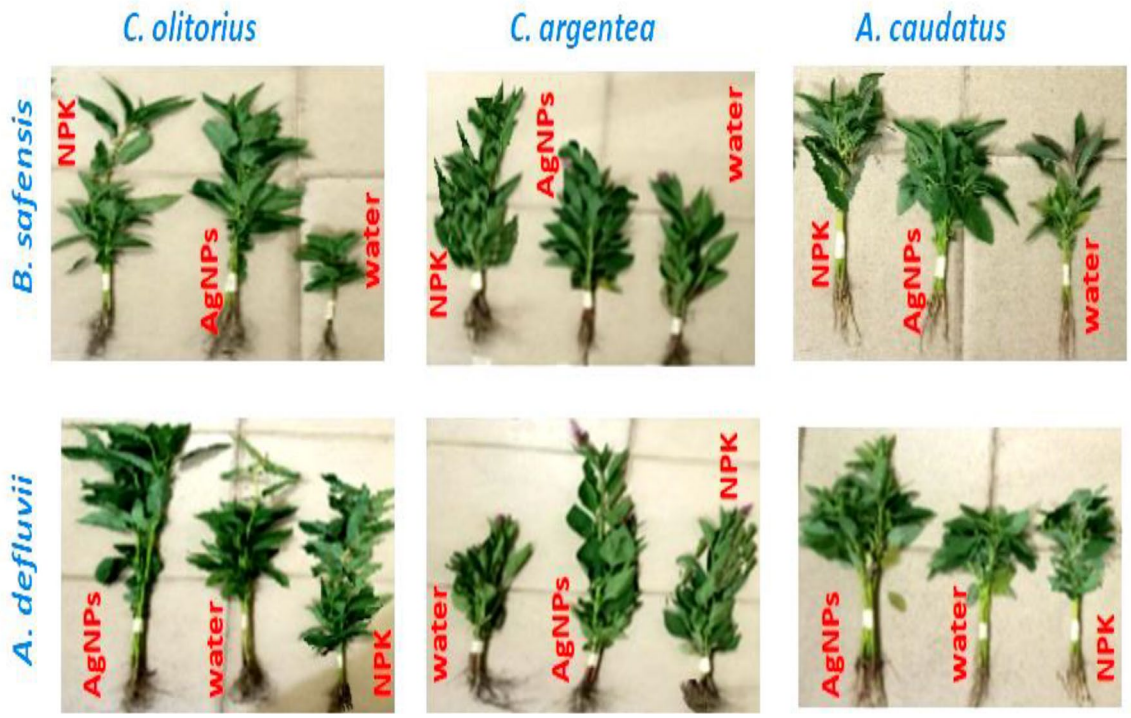


Fig. 5 Plant growth-promoting effects of the biosynthesized silver nanoparticles (AgNPs) (150 µg/ml) on growth of *Corchorus olitorius*, *Celosea argentea* and *Amarantus caudatus* after 5 weeks in pot conditions

Table 1 Effects of FH-AgNPs mediated by *B. safensis* feather hydrolysate on growth and photosynthetic parameters of vegetables

Treatment	SH (cm)	RL (cm)	SFW (g)	SDW (g)	GRI	LN	LA (cm^2)	CL (mg/g FW)
Corchorus olitorius								
Water	11.67 ± 0.77^{d}	7.30 ± 0.66^{d}	5.10 ± 0.35^{c}	1.03 ± 0.03^{d}	19.05 ± 0.64^{b}	4.67 ± 0.29^{c}	3.26 ± 0.18^{e}	1.09 ± 0.31^{e}
NPK	30.78 ± 0.67^{bc}	11.45 ± 0.35^{bc}	9.18 ± 0.31^{b}	1.81 ± 0.01^{c}	21.28 ± 0.54^{ab}	6.87 ± 0.29^{b}	9.23 ± 1.72^{c}	1.27 ± 0.06^{c}
150 (µg/ml)	38.54 ± 1.78^{a}	15.44 ± 0.92^{a}	11.77 ± 1.20^{a}	2.71 ± 0.22^{a}	22.33 ± 0.81^{a}	8.67 ± 1.04^{a}	15.32 ± 1.01^{a}	1.98 ± 0.16^{a}
100 (µg/ml)	33.89 ± 0.48^{b}	12.22 ± 0.26^{b}	10.14 ± 0.08^{ab}	2.23 ± 0.10^{b}	22.00 ± 0.66^{a}	7.67 ± 0.58^{ab}	11.00 ± 1.15^{b}	1.31 ± 0.21^{b}
50 (µg/ml)	23.78 ± 1.02^{c}	10.11 ± 0.39^{c}	5.10 ± 0.36^{c}	1.82 ± 0.02^{c}	19.67 ± 0.72^{b}	5.67 ± 0.29^{bc}	6.52 ± 0.19^{d}	1.19 ± 0.22^{d}
Celosia argentea								
Water	27.65 ± 1.66^{b}	9.32 ± 1.02^{a}	26.25 ± 1.61^{a}	4.69 ± 0.31^{b}	13.87 ± 1.42^{a}	09.03 ± 0.65^{ab}	9.12 ± 0.99^{a}	1.26 ± 0.21^{b}
NPK	32.05 ± 1.01^{a}	10.34 ± 0.49^{a}	27.35 ± 1.06^{a}	5.13 ± 0.23^{a}	14.39 ± 1.16^{a}	10.35 ± 0.54^{a}	10.63 ± 0.78^{a}	1.32 ± 0.16^{a}
150 (µg/ml)	29.75 ± 1.19^{ab}	10.11 ± 0.56^{a}	27.28 ± 1.51^{a}	5.02 ± 0.29^{a}	14.06 ± 1.81^{a}	10.29 ± 0.68^{a}	9.74 ± 1.02^{a}	1.32 ± 0.31^{a}
100 (µg/ml)	24.77 ± 1.58^{bc}	9.11 ± 0.66^{a}	22.34 ± 1.31^{b}	4.53 ± 0.22^{b}	13.06 ± 1.81^{a}	8.57 ± 0.32^{b}	8.79 ± 1.06^{a}	1.25 ± 0.26^{b}
50 (µg/ml)	24.15 ± 1.57^{bc}	8.84 ± 0.62^{a}	21.94 ± 0.67^{b}	4.48 ± 0.19^{b}	13.01 ± 1.36^{a}	8.49 ± 0.22^{b}	8.79 ± 0.84^{a}	1.23 ± 0.11^{bc}
Amaranthus caudatus								
Water	26.01 ± 1.61^{a}	9.79 ± 1.38^{a}	26.64 ± 1.88^{b}	6.42 ± 0.55^{c}	13.03 ± 0.81^{ab}	9.08 ± 0.61^{a}	13.03 ± 1.37^{b}	0.84 ± 0.14^{c}
NPK	26.13 ± 1.28^{a}	10.23 ± 1.01^{a}	27.16 ± 1.17^{b}	7.13 ± 0.31^{b}	13.89 ± 0.98^{ab}	9.10 ± 0.28^{a}	13.09 ± 1.02^{b}	0.98 ± 0.04^{b}
150 (µg/ml)	26.75 ± 1.08^{a}	10.78 ± 1.23^{a}	32.16 ± 1.48^{a}	9.02 ± 1.06^{a}	15.31 ± 0.58^{a}	10.03 ± 0.37^{a}	16.44 ± 1.20^{a}	1.09 ± 0.19^{a}
100 (µg/ml)	23.65 ± 1.56^{b}	9.11 ± 1.06^{a}	25.21 ± 1.83^{b}	6.01 ± 1.03^{c}	12.54 ± 0.35^{b}	6.35 ± 0.88^{b}	12.59 ± 0.99^{b}	0.73 ± 0.06^{d}
50 (µg/ml)	23.03 ± 1.33^{b}	9.02 ± 1.19^{a}	24.98 ± 2.48^{b}	5.97 ± 0.39^{d}	12.54 ± 0.35^{b}	6.17 ± 0.82^{b}	12.48 ± 0.88^{b}	0.70 ± 0.07^{d}

SH shoot height, *RL* root length, *SFW* shoot fresh weight, *SDW* shoot dry weight, *GRI* germination rate index, *LN* leaf number, *LA* leaf area, *CL* chlorophyll. Values are mean ± standard error of mean for four replicates. Data with the same superscript within a column are not significantly different ($p < 0.05$)

had highest SH (38.54 cm), RL (15.44 cm), GRI (22.33%) compared to 11.67 cm, 7.30 cm and 19.05%, respectively, obtained in the control plant. The FH-AgNPs also enhanced the germination, shoot height, root length and other growth parameters of *A. caudatus* and *C. argentea* seeds than their controls by more than 1.0-fold. The FH-AgNPs (150 µg/ml) produced higher growth-promoting activities in all the vegetables than other treatments except in *Celosia argentea* that the application of NPK fertilizer performed better.

The AgNPs synthesized from *Aquamicrobium defluvii* FH produced similar growth-promoting effect on the vegetables. Their seeds germination was boosted upon treatment with the FH-AgNPs (150 µg/ml) such that GRI with maximum values of 22.98, 15.50, and 15.44% was obtained for *Corchorus olitorius*, *Celosia argentea* and *Amaranthus caudatus* seeds compared to 19.67, 9.97 and 13.03% recorded, respectively, for their controls (Table 2). The particles remarkably enhanced the vegetable growth as higher shoot height with values of 39.01, 44.78 and 28.76 cm were obtained for *Corchorus olitorius*, *Celosia argentea* and *Amaranthus caudatus* plants, respectively, corresponding to 1.58, 1.52, 1.58-fold improvement over their controls. The AgNPs also produced better effects on other parameters such as root length, shoot fresh weight, shoot dry weight, leaf size and chlorophyll than their respective control plants. It was observed that activity of the particles increases with the concentration and the optimum was produced by the highest dose(150 µg/ml). The FH-AgNPs at optimal dose produced better impact on the vegetable seeds germination and plant growth.

Measurement of parameters such as seed germination, plant height, root length and leaf size is often used to assess the effect of nanoparticles on plant growth. Phytostimulatory effects of biosynthesized AgNPs on the seed germination, seedling growth and biomass yield of various plants have been reported [20, 51]. Tung et al. [52] reported that the application of 7.5 ppm AgNPs to the tissue culture of *Chrysanthemum morifolium* significantly improved the plant height, leaf sizes, plant fresh and dry weight. Most importantly, the AgNPs increased the plant height from 3 to 6.98 cm during the 2 weeks of cultivation. Similarly, AgNPs application at 100 ppm dosage enhanced leaf and bulb biomass, total chlorophyll and flower abundance of two cultivars of oriental lily [53]. However, the plant growth-promoting mechanisms of nanoparticles are not well known, their possible mode of actions has been suggested. For instance, the positive effect of the biogenic AgNPs on growth of the vegetables as obtained herein could be ascribed to its efficacy to enhance the activities of enzymes needed for improved seed germination and seedling growth [54]. Also, AgNPs can stimulate complex physiological processes like alteration of expression of genes that control multiple cellular pathways, including cell proliferation, photosynthesis and hormone-signaling pathways, such as indolic acid, abscisic acid and ethylene [55].

Table 2 Effect of FH-AgNPs mediated by *A. defluvii* feather hydrolysate on growth and photosynthetic parameters of vegetables

Treatment	SH (cm)	RL (cm)	SFW (g)	SDW (g)	GRI	LN	LA (cm^2)	CL (mg/g FW)
Corchorus olitorius								
Water	24.75 ± 1.21^{c}	10.95 ± 0.51^{c}	6.01 ± 0.96^{c}	1.74 ± 0.14^{c}	19.67 ± 0.72^{b}	6.11 ± 0.77^{b}	10.06 ± 0.64^{b}	1.23 ± 0.21^{d}
NPK	24.80 ± 0.63^{c}	10.99 ± 0.45^{c}	6.03 ± 1.02^{c}	1.74 ± 0.05^{c}	20.15 ± 0.72^{b}	6.11 ± 0.96^{b}	10.19 ± 0.66^{b}	1.29 ± 0.11^{c}
150 (µg/ml)	39.01 ± 1.61^{a}	15.45 ± 1.01^{a}	12.11 ± 0.88^{a}	2.96 ± 0.61^{a}	22.98 ± 0.61^{a}	8.97 ± 0.99^{a}	15.44 ± 0.93^{a}	1.85 ± 0.22^{a}
100 (µg/ml)	31.43 ± 0.82^{b}	12.01 ± 0.36^{b}	10.01 ± 1.07^{b}	2.05 ± 0.05^{b}	20.18 ± 0.67^{b}	6.87 ± 0.66^{b}	10.73 ± 1.01^{b}	1.39 ± 0.28^{b}
50 (µg/ml)	20.22 ± 0.91^{cd}	10.02 ± 0.27^{c}	4.76 ± 0.51^{d}	1.65 ± 0.02^{c}	17.68 ± 0.67^{c}	5.12 ± 0.32^{c}	10.02 ± 0.65^{b}	1.21 ± 0.17^{d}
Celosia argentea								
Water	29.44 ± 2.08^{c}	10.0 ± 1.01^{a}	24.73 ± 2.36^{c}	4.67 ± 0.39^{b}	9.97 ± 0.26^{b}	9.67 ± 0.29^{b}	13.57 ± 1.41^{ab}	1.18 ± 0.21^{b}
NPK	31.55 ± 1.17^{bc}	10.05 ± 0.54^{a}	27.04 ± 1.10^{b}	5.04 ± 0.07^{b}	12.93 ± 1.61^{ab}	10.33 ± 0.77^{b}	14.12 ± 1.04^{ab}	1.19 ± 0.16^{b}
150 (µg/ml)	44.78 ± 1.59^{a}	12.22 ± 0.39^{a}	41.10 ± 0.65^{a}	6.79 ± 0.01^{a}	15.50 ± 0.77^{a}	12.67 ± 0.29^{a}	16.65 ± 1.57^{a}	1.52 ± 0.20^{a}
100 (µg/ml)	35.11 ± 1.01^{b}	11.44 ± 0.59^{a}	27.98 ± 0.03^{b}	5.49 ± 0.04^{b}	14.06 ± 1.85^{ab}	12.33 ± 0.58^{a}	15.44 ± 1.80^{a}	1.22 ± 0.09^{b}
50 (µg/ml)	35.00 ± 2.85^{b}	10.89 ± 0.67^{a}	27.10 ± 3.09^{b}	5.42 ± 0.24^{b}	13.87 ± 0.42^{ab}	12.13 ± 0.29^{a}	15.31 ± 1.07^{b}	1.22 ± 0.11^{b}
Amaranthus caudatus								
Water	18.17 ± 1.85^{b}	7.90 ± 1.47^{b}	25.15 ± 1.19^{b}	5.81 ± 0.36^{b}	13.03 ± 0.25^{ab}	8.03 ± 0.29^{ab}	10.01 ± 0.61^{bc}	0.90 ± 0.11^{b}
NPK	19.00 ± 1.01^{b}	8.22 ± 1.51^{b}	26.23 ± 1.94^{b}	5.97 ± 0.49^{b}	14.37 ± 0.90^{a}	8.67 ± 0.29^{ab}	13.04 ± 1.60^{b}	0.95 ± 0.05^{b}
150 (µg/ml)	28.76 ± 2.50^{a}	10.78 ± 1.35^{a}	34.18 ± 5.49^{a}	9.88 ± 1.12^{a}	15.44 ± 0.86^{a}	10.33 ± 0.77^{a}	16.50 ± 1.73^{a}	1.09 ± 0.09^{a}
100 (µg/ml)	18.33 ± 2.09^{b}	8.19 ± 1.19^{b}	25.72 ± 3.45^{b}	5.88 ± 0.75^{b}	14.35 ± 0.89^{a}	8.33 ± 0.50^{ab}	10.36 ± 1.07^{bc}	0.95 ± 0.07^{b}
50 (µg/ml)	16.56 ± 2.36^{b}	7.33 ± 1.02^{b}	21.62 ± 4.11^{c}	4.86 ± 0.94^{c}	12.54 ± 0.35^{ab}	6.67 ± 0.58^{b}	08.20 ± 0.19^{c}	0.87 ± 0.13^{bc}

SH shoot height, *RL* root length, *SFW* shoot fresh weight, *SDW* shoot dry weight, *GRI* germination rate index, *LN* leaf number, *LA* leaf area, *CL* chlorophyll. Values are mean ± standard error of mean for four replicates. Data with the same superscript within a column are not significantly different ($p < 0.05$)

Furthermore, Azeez et al. [11] reported a similar plant growth-promoting effect of biogenic AgNPs as the particles elongated the shoot and root length of *C. olitorius* cultivated under pot conditions in the range of 7.93–9.65 cm and 2.63 to 3.62 cm, respectively. The increase in root and shoot lengths mediated by AgNPs could be due to the ability of AgNPs to enhance plant regulators required for cell divisions, cell elongation as well as enlargement of root pores which in turn stimulate nutrients and mineral uptake [56]. Large surface area of nanoparticles facilitates their interaction with nutrients in the rhizosphere, and their high penetrating power promotes the translocation of nutrients from the root region to the aerial parts of plant [14, 57]. Nanoparticles boost the photosynthetic process of plants as they increase the absorption and translocation of nutrients involved in chlorophyll formation [21]. Conversely, inconsistent plant responses to AgNPs have been reported which could be attributed to variation in plant genotype, AgNPs concentration and mode of application [53]. Other factors, such as size, morphology, chemical compositions and mode of synthesis, also make it difficult to compare the results of different studies and to explain the mechanisms of AgNPs action. In addition, richness of the FHs in nutrients, amino acids and soluble proteins could have contributed immensely to the plant-growth-promoting property demonstrated by the biogenic FH-AgNPs. The nutrients and amino acids have been found capable of enhancing soil fertility and microbial activity which consequently stimulate plant growth and metabolism [31].

Antioxidant activities of vegetables grown with FH-AgNPs

The biosynthesized AgNPs from feather hydrolysate of *B. safensis* improved the hydrogen peroxide and DPPH radical scavenging activities of the vegetables. The free radicals scavenging activity of the vegetables increased with the concentration of FH-AgNPs with maximum activity been produced by 150 µg/ml concentration (Table 3). Extract (1 mg/ml) of *Corchorus olitorius, Celosia argentea* and *Amaranthus caudatus* grown with FH-AgNPs (150 µg/ml) scavenged 63.89, 66.21, 61.89% hydrogen peroxide compared to 56.94, 54.42, 55.33% recorded for their controls (water), respectively. In the same vein, DPPH free radical scavenging activities with maximum values of 48.63, 44.76 and 44.13% were recorded for *C. olitorius, C. argentea* and *A. caudatus* treated with FH-AgNPs (150 µg/ml) compared to 25.74, 39.34 and 41.06% obtained from their control plants, respectively.

Table 3 Antioxidant activities and lipid peroxidation inhibition effect of vegetables grown with FH-AgNPs produced by *B. safensis* FH

Extract	% H_2O_2 scavenged	% DPPH scavenged	% LPO inhibition	Catalase activities (unit/mg protein)
C. olitorius				
Water	56.94 ± 2.95^{d}	25.74 ± 0.25^{d}	72.98 ± 1.38^{c}	283.20 ± 0.50^{c}
NPK	58.33 ± 0.57^{c}	43.58 ± 0.28^{b}	74.52 ± 1.02^{c}	301.06 ± 0.71^{b}
150 (µg/ml)	63.89 ± 0.05^{b}	48.63 ± 0.28^{a}	76.86 ± 0.72^{b}	309.83 ± 0.36^{a}
100 (µg/ml)	59.72 ± 0.98^{c}	34.51 ± 0.42^{c}	73.21 ± 1.22^{c}	292.00 ± 0.07^{b}
50 (µg/ml)	58.12 ± 0.61^{c}	33.99 ± 0.23^{c}	73.17 ± 0.40^{c}	274.38 ± 0.38^{d}
Ascorbic acid	66.67 ± 1.97^{a}	47.83 ± 0.07^{a}	80.30 ± 1.30^{a}	289.69 ± 0.38^{b}
C. argentea				
Water	54.42 ± 0.59^{d}	39.34 ± 0.53^{c}	71.33 ± 1.07^{b}	270.52 ± 1.14^{d}
NPK	63.54 ± 0.38^{b}	42.83 ± 0.42^{b}	78.03 ± 0.54^{a}	276.58 ± 0.67^{c}
150 (µg/ml)	66.21 ± 0.17^{a}	44.76 ± 0.62^{b}	79.68 ± 1.16^{a}	283.11 ± 1.37^{b}
100 (µg/ml)	61.31 ± 0.41^{c}	39.41 ± 0.38^{c}	66.71 ± 0.67^{c}	272.33 ± 0.73^{d}
50 (µg/ml)	60.53 ± 0.25^{c}	38.11 ± 0.57^{c}	65.89 ± 0.37^{c}	269.88 ± 0.59^{d}
Ascorbic acid	66.67 ± 1.97^{a}	47.83 ± 0.07^{a}	80.30 ± 1.30^{a}	289.69 ± 0.38^{a}
A. caudatus				
Water	55.33 ± 0.82^{c}	41.06 ± 0.37^{c}	70.41 ± 0.61^{c}	254.22 ± 0.29^{d}
NPK	59.12 ± 0.49^{b}	44.10 ± 0.17^{b}	73.19 ± 0.76^{b}	261.31 ± 0.37^{c}
150 (µg/ml)	61.89 ± 0.67^{b}	44.13 ± 0.33^{b}	78.11 ± 0.91^{a}	275.45 ± 0.51^{b}
100 (µg/ml)	58.12 ± 0.61^{bc}	43.21 ± 0.11^{b}	71.36 ± 1.02^{b}	260.91 ± 0.46^{c}
50 (µg/ml)	56.01 ± 0.39^{c}	40.17 ± 0.27^{c}	69.84 ± 0.69^{c}	260.23 ± 0.32^{c}
Ascorbic acid	66.67 ± 1.97^{a}	47.83 ± 0.07^{a}	80.30 ± 1.30^{a}	289.69 ± 0.38^{a}

Values are mean ± standard error of mean for four replicates. Data with the same superscript within a column are not significantly different ($p < 0.05$)

Similar results were obtained on the antioxidant activity of the vegetables cultivated with biosynthesized AgNPs from feather hydrolysate of *A. defluvii*. Highest hydrogen peroxide scavenging activity with values of 64.76, 62.50 and 59.72% were produced by FH-AgNPs-treated *C. olitorius, C. argentea* and *A. caudatus* compared to 61.03, 55.77 and 48.61% recorded for their control plants, respectively (Table 4). More so, treatment with optimal dose of FH-AgNPs (150 µg/ml) enhanced the potency of the vegetables to scavenge DPPH free radicals as they exhibited over 1.1-fold improvement in activity than their control water cultivated plants. Generally, it was observed that AgNPs at 100–150 µg/ml produced better effect by elevating the antioxidant activities of the vegetables compared to water grown plants. At optimal dose, the biosynthesized FH-AgNPs performed considerably better than the NPK fertilizer.

Tissue damage occurs in living organisms when there is imbalance between the production of free radicals from food metabolism and their removal by antioxidant defense systems [58]. Tissue damage by free radicals may result to diseases like cancer, cardiovascular disease, diabetes, cataracts, immune system decline, liver diseases, inflammation, renal failure, brain dysfunction and stress among others [59]. Thus, it is very necessary to consume foods rich in antioxidants such as fruits and vegetables to boost the free radical scavenging systems in the body. Enhancement of antioxidant properties of some important plants has been documented using biological approaches. For instance, Azeez et al. [11] reported a very significant improvement in the antioxidant property of *C. olitorius* grown with biosynthesized AgNPs. Foliar application of metallic nanoparticles was reported to significantly increase the antioxidant effect of oak leaf lettuce seedlings, such that the DPPH radicals scavenging activities of the AgNPs and platinum nanoparticles (Pt-NPs)-treated oak leaf lettuce extracts were 37.5 and 44% higher than the controls, respectively [60]. Similarly, foliar spray of *Echium amoenum* seedlings with 50 ppm AgNPs was reported to significantly improve its potency to scavenge DPPH radicals [61]. Nanoparticles have been indicated of being capable of suppressing oxidative stress in plant through their ability to activate the antioxidative defense system consisting of superoxide dismutase (SOD), catalase, peroxidase and the ascorbate glutathione enzymes [62].

Phytochemical compositions of vegetables grown with the biosynthesized FH-AgNPs

Application of biosynthesized FH-AgNPs as nanofertilizer produced a positive effect on cultivation of the vegetables as the contents of their phytochemicals compounds were

Table 4 Antioxidant activities and lipid peroxidation inhibition effect of vegetables grown with FH-AgNPs produced by *A. defluvii* FH

Extract	% H_2O_2 scavenged	% DPPH scavenged	% LPO inhibition	Catalase activities (unit/mg protein)
C. olitorius				
Water	61.03 ± 0.56^{b}	37.85 ± 0.29^{d}	73.11 ± 0.87^{b}	284.16 ± 0.63^{d}
NPK	61.13 ± 0.32^{b}	45.23 ± 0.16^{b}	77.56 ± 0.45^{a}	293.56 ± 0.33^{b}
150 (µg/ml)	64.76 ± 0.51^{a}	47.97 ± 0.24^{a}	78.14 ± 0.61^{a}	297.13 ± 1.06^{a}
100 (µg/ml)	60.55 ± 0.24^{b}	45.22 ± 0.48^{b}	75.74 ± 1.08^{a}	291.56 ± 0.84^{b}
50 (µg/ml)	58.73 ± 0.17^{c}	41.13 ± 0.24^{c}	70.49 ± 0.77^{c}	287.78 ± 1.07^{c}
Ascorbic acid	66.67 ± 1.97^{a}	47.83 ± 0.07^{a}	80.30 ± 1.29^{a}	289.69 ± 0.38^{c}
C. argentea				
Water	55.77 ± 0.45^{d}	39.85 ± 0.11^{b}	68.29 ± 1.57^{c}	262.34 ± 0.60^{d}
NPK	59.72 ± 0.98^{c}	41.52 ± 0.25^{b}	73.28 ± 0.68^{b}	263.75 ± 0.02^{d}
150 (µg/ml)	62.50 ± 0.98^{b}	42.50 ± 0.12^{b}	81.01 ± 1.20^{a}	281.72 ± 1.68^{b}
100 (µg/ml)	56.94 ± 0.98^{d}	40.78 ± 0.07^{b}	69.49 ± 1.22^{c}	270.76 ± 0.75^{c}
50 (µg/ml)	55.02 ± 0.89^{d}	38.75 ± 0.19^{b}	68.34 ± 0.79^{c}	270.02 ± 0.27^{c}
Ascorbic acid	66.67 ± 1.97^{a}	47.83 ± 0.07^{a}	80.30 ± 1.30^{a}	289.69 ± 0.38^{a}
A. caudatus				
Water	48.61 ± 0.96^{d}	36.77 ± 0.11^{c}	63.51 ± 0.90^{e}	236.79 ± 0.18^{d}
NPK	56.94 ± 0.98^{c}	40.03 ± 0.18^{b}	73.06 ± 1.07^{c}	259.85 ± 0.42^{b}
150 (µg/ml)	59.72 ± 0.87^{b}	40.85 ± 0.28^{b}	76.96 ± 1.02^{b}	263.11 ± 0.17^{b}
100 (µg/ml)	50.71 ± 1.97^{d}	39.43 ± 0.04^{b}	73.11 ± 0.69^{c}	252.02 ± 0.32^{c}
50 (µg/ml)	50.11 ± 0.58^{d}	35.73 ± 0.36^{c}	70.36 ± 1.02^{d}	239.31 ± 0.36^{d}
Ascorbic acid	66.67 ± 1.97^{a}	47.83 ± 0.07^{a}	80.30 ± 1.30^{a}	289.69 ± 0.38^{a}

Values are mean ± standard error of mean for four replicates. Data with the same superscript within a column are not significantly different ($p < 0.05$)

Table 5 Phytochemical constituents of vegetables grown with FH-AgNPs synthesized using *B. safensis* LAU 13 feather hydrolysate

Treatments	Flavonoids (mg/g of catechin)	Total phenols (mg/g of gallic acid)	Total Proanthocyanidin (mg/g of catechin)
C. olitorius			
Water	53.11 ± 0.51^{a}	221.15 ± 3.80^{b}	98.76 ± 1.03^{a}
NPK	55.12 ± 0.33^{a}	228.42 ± 2.01^{b}	101.23 ± 0.57^{a}
150 (µg/ml)	55.74 ± 0.16^{a}	245.13 ± 3.62^{a}	105.34 ± 1.59^{a}
100 (µg/ml)	54.96 ± 0.57^{a}	240.74 ± 2.93^{a}	100.27 ± 1.61^{a}
50 (µg/ml)	54.10 ± 0.66^{a}	201.33 ± 2.56^{c}	97.17 ± 1.58^{a}
C. argentea			
Water	55.11 ± 0.71^{b}	240.89 ± 2.72^{b}	73.45 ± 3.06^{c}
NPK	55.84 ± 0.51^{b}	244.32 ± 2.53^{b}	79.21 ± 2.34^{b}
150 (µg/ml)	60.75 ± 0.81^{a}	263.15 ± 2.37^{a}	85.33 ± 2.18^{a}
100 (µg/ml)	54.89 ± 0.66^{b}	238.21 ± 2.51^{b}	71.99 ± 1.83^{c}
50 (µg/ml)	45.94 ± 0.33^{c}	235.78 ± 2.28^{b}	71.35 ± 1.72^{c}
A. caudatus			
Water	60.31 ± 1.07^{b}	269.45 ± 3.09^{b}	119.16 ± 2.19^{b}
NPK	64.85 ± 0.66^{a}	280.34 ± 1.81^{a}	122.6 ± 1.08^{b}
150 (µg/ml)	65.74 ± 1.03^{a}	283.62 ± 2.67^{a}	127.13 ± 1.95^{a}
100 (µg/ml)	60.15 ± 0.88^{b}	280.13 ± 2.60^{a}	109.73 ± 2.02^{c}
50 (µg/ml)	58.67 ± 0.63^{b}	265.76 ± 3.64^{b}	108.28 ± 1.59^{c}

Values are mean ± standard error of mean for four replicates. Data with the same superscript within a column are not significantly different ($p < 0.05$)

Table 6 Phytochemical constituents of vegetables grown with FH-AgNPs synthesized using *A. defluvii* FH 20 feather hydrolysate

Treatments	Flavonoids (mg/g of catechin)	Total phenols (mg/g of gallic acid)	Total Proanthocyanidin (mg/g of catechin)
C. olitorius			
Water	56.98 ± 1.05^{a}	280.37 ± 4.06^{c}	109.96 ± 2.06^{b}
NPK	58.76 ± 0.57^{a}	291.14 ± 2.91^{b}	112.83 ± 1.09^{b}
150 (µg/ml)	61.32 ± 1.02^{a}	305.22 ± 3.12^{a}	119.62 ± 1.33^{a}
100 (µg/ml)	56.81 ± 0.62^{a}	293.72 ± 2.67^{b}	110.55 ± 0.67^{b}
50 (µg/ml)	56.12 ± 0.83^{a}	271.48 ± 3.02^{d}	103.41 ± 1.45^{c}
C. argentea			
Water	50.76 ± 0.55^{b}	270.95 ± 2.61^{b}	102.24 ± 3.01^{c}
NPK	52.46 ± 0.33^{b}	274.45 ± 2.38^{b}	115.38 ± 1.65^{b}
150 (µg/ml)	59.67 ± 0.26^{a}	296.13 ± 2.57^{a}	121.34 ± 2.06^{a}
100 (µg/ml)	51.14 ± 0.38^{b}	291.77 ± 3.02^{a}	112.67 ± 1.73^{b}
50 (µg/ml)	43.15 ± 0.52^{c}	272.46 ± 2.92^{b}	91.76 ± 1.94^{d}
A. caudatus			
Water	58.05 ± 0.43^{b}	290.65 ± 4.02^{b}	109.20 ± 3.62^{b}
NPK	65.92 ± 0.19^{a}	294.17 ± 2.16^{b}	112.93 ± 2.07^{b}
150 (µg/ml)	66.85 ± 0.37^{a}	304.26 ± 2.81^{a}	125.16 ± 1.82^{a}
100 (µg/ml)	59.11 ± 0.59^{b}	290.55 ± 4.86^{b}	108.73 ± 3.12^{b}
50 (µg/ml)	51.04 ± 0.44^{c}	283.19 ± 2.28^{c}	103.75 ± 2.59^{c}

Values are mean ± standard error of mean for four replicates. Data with the same superscript within a column are not significantly different ($p < 0.05$)

considerably increased as shown in Table 5. The total phenolic, flavonoids and proanthocyanidin compounds found in the three vegetables grown with the *B. safensis* AgNPs increased with the concentration of the AgNPs. *C. olitorius* treated with FH-AgNPs (150 µg/ml) had maximum amount of total phenols (245.13 mg GAE/g), flavonoids (55.74 mg CE/g) and total proanthocyanidin (105.34 mg CE/g) representing 1.11, 1.05 and 1.07-fold improvement compared to control (water), respectively. Furthermore, the FH-AgNPs at optimal dose (150 µg/ml) increased the total phenols, flavonoids and total proanthocyanidin in *C. argentea* and *A. caudatus* by more than 1.1-fold compared to control. Also, the same treatment enriched the phytochemical contents of the vegetables than the positive control (NPK fertilizer).

Similarly, the biogenic FH-AgNPs of *A. defluvii* FH enhanced the contents of phytochemical compounds in the vegetables. The activity of AgNPs at 150 µg/ml was found to be optimal as it produced *C. olitorius* with highest contents of phenols (305.22 mg GAE/g), flavonoids (61.32 mg CE/g) and total proanthocyanidin (119.62 mg CE/g) representing 1.09, 1.08 and 1.09-fold improvement compared to control (water), respectively (Table 6). Moreover, the proanthocyanidin and flavonoids contents in *C. argentea* and *A. caudatus* were increased by about 1.15-fold after treatment with FH-AgNPs (150 µg/ml), while approximately 1.05-fold improvement was recorded for total phenolic contents in the both vegetables over their respective water-treated control plants. The FH-AgNPs also induced above 1.0-fold improvement in the phytochemicals contents of the three vegetables compared to NPK-treated plants. Generally, it was observed that the amount of phytochemical compounds in the vegetables increased with the concentration of biogenic FH-AgNPs indicating dose-dependent activities.

Phytochemicals provide desirable health benefits beyond basic nutrition to reduce the risk of some chronic diseases. High concentrations of phytochemicals in food plants may protect against free radical damage in living tissues [63]. There exists a positive correlation between antioxidant activity and phytochemicals contents of vegetables. Antioxidants protect human body through mechanisms like inhibition of free radicals generation, interception of radical-chained reactions, conversion of free radicals into less harmful molecules and repair of oxidative damage [64]. Regular intake of fruits and vegetables as foods rich in natural antioxidant molecules is encouraged to protect body from deleterious effects of free radicals as studies have shown that their consumption has positive correlation to the prevention of degenerative diseases [65]. Moreover, sustainable enhancement of phytochemical contents of food plants is suggested so as to boost their antioxidant efficacy in the body. Thus, the level of

improvement in the phytochemical contents of vegetables using nanobiotechnological approach as obtained herein corresponds with the findings of Azeez et al. [10] that reported significant improvement in the quantity of phenolic and flavonoid contents of *A. caudatus* cultivated with biogenic AgNPs compared to water grown control plant. Therefore, it can be inferred that nanobiotechnology could be deployed as a promising and sustainable technology to boost the natural antioxidant compounds in food plants.

Lipid peroxidation inhibition and catalase activities of vegetables grown with biosynthesized FH-AgNPs

Application of biosynthesized FH-AgNPs as nanofertilizers enhanced the lipid peroxidation inhibition effect of the vegetables as they induce inhibition of lipid peroxidation against ferrous ion (Fe^{2+}) damage in the precision-cut liver slices (PCLS). Highest LPO inhibition of 76.86, 79.68 and 78.11% was obtained for *C. olitorius, C. argentea* and *A. caudatus* (Table 3) grown with 150 µg/ml FH-AgNPs produced from *B. safensis* FH, compared to 72.98, 71.33, 70.41% recorded for the water-treated plants (control), respectively. Activity of the FH-AgNPs is dose dependent as the maximum LPO inhibition was recorded for the vegetable cultivated with highest dose (150 µg/ml). Similarly, FH-AgNPs biosynthesized from *A. defluvii* FH at optimal concentration produced *C. olitorius, C. argentea* and *A. caudatus* whose extracts demonstrated LPO inhibition better than lower concentrations and their controls (Table 4). The FH at the concentration of 100–150 µg/ml induced over 1.0-fold improvement in LPO inhibitory activity of the three vegetables. Generally, *C. olitorius, C. argentea* and *A. caudatus* fertilized with *B. safensis* and *A. defluvii* AgNPs exhibited LPO inhibitory activity by more than 1.01-fold over NPK-fertilized plants. Comparatively, it was observed that the three vegetables treated with the biosynthesized FH-AgNPs at optimal dose demonstrated LPO inhibitory activity approximately equal to ascorbic acid used as standard.

Free radicals induce lipid peroxidation, a process that occurs when biomolecules like lipids, proteins and nucleic acids are oxidized by radicals to cause severe diseases such as cancer, cardiovascular and neurodegenerative disorder [66]. Consumption of healthy foods like fruits and vegetables that are rich in antioxidant molecules is often advocated to safeguard excessive formation of free radicals in the body so as to reduce oxidative damage and lipid peroxidation [67]. Phytochemicals like phenols and flavonoids have been reported as potent antioxidant molecules capable of scavenging free radicals to prevent tissue damage and risk of cardiovascular disorder. High lipid peroxidation inhibitions exhibited by AgNPs-treated vegetables under this investigation could be ascribed to their richness in antioxidants molecules such as phenolic and flavonoid compounds. This result is in tandem with the findings of Azeez et al. [11] that reported a significant LPO inhibition against hydrogen peroxide challenge in PCLS by aqueous extract of *C. olitorius* cultivated with biosynthesized AgNPs. Hence, the ability of the vegetables cultivated with the biosynthesized AgNPs to attenuate liver cell damage induced by ferrous ion suggests promising application of nanobiotechnology as a sustainable tool to boost nutraceutical properties of vegetables.

Application of biosynthesized FH-AgNPs as nanofertilizers remarkably enhanced catalase activity of the vegetables. The AgNPs influenced the catalase activity of the vegetables in dose-dependent manners as the optimal activity was recorded for those cultivated with highest concentration (150 µg/ml). Vegetables grown with 100–150 µg/ml FH-AgNPs produced from *B. safensis* FH exhibited higher catalase activity than the water-treated control plants (Table 3). The *Corchorus olitorius, Celosia argentea* and *Amaranthus caudatus* cultivated with 150 µg/ml FH-AgNPs produced optimum activity of 309.83, 283.11 and 275.45 unit/mg protein, which is 1.09, 1.05 and 1.08-fold higher than water-treated plants, respectively. Vegetables grown with 150 µg/ml AgNPs produced better catalase activity than NPK-treated plants.

More so, *A. defluvii* FH-AgNPs demonstrated positive effect on the catalase activity of the three vegetables. The particles at all concentrations (50, 100, and 150 µg/ml) influenced catalase activity in the vegetables better than the control plants in dose-dependent manners (Table 4). At optimal dose, activity of all vegetables treated with FH-AgNPs was better than control plants by more than 1.03-fold. They also displayed better catalase activity than the positive control (NPK fertilizer). It was observed that the biosynthesized FH-AgNPs in most cases compared moderately with ascorbic acid used as standard.

Peroxides are continuously generated as by-products of metabolic reactions, and their accumulation induces severe damage to cells and tissue in the body. However, they are naturally removed from body by catalase enzyme produced in the mammalian liver. Catalase as an antioxidant enzyme removes peroxides from the body by converting them into water and oxygen to safeguard tissue damage from their hydroxyl radicals' attack [68]. Thus, consumption of diet rich in catalase concentration is encouraged to attenuate hydroxyl radical activity. The ability of vegetables cultivated with the biosynthesized AgNPs from the bacterial FHs to display potent catalase activity in the liver homogenate as obtained in this study could be attributed to their high phenolic and flavonoid contents. Hence, application of nanobiotechnology as a sustainable approach to boost the antioxidant efficacy of healthy agricultural foods is suggested.

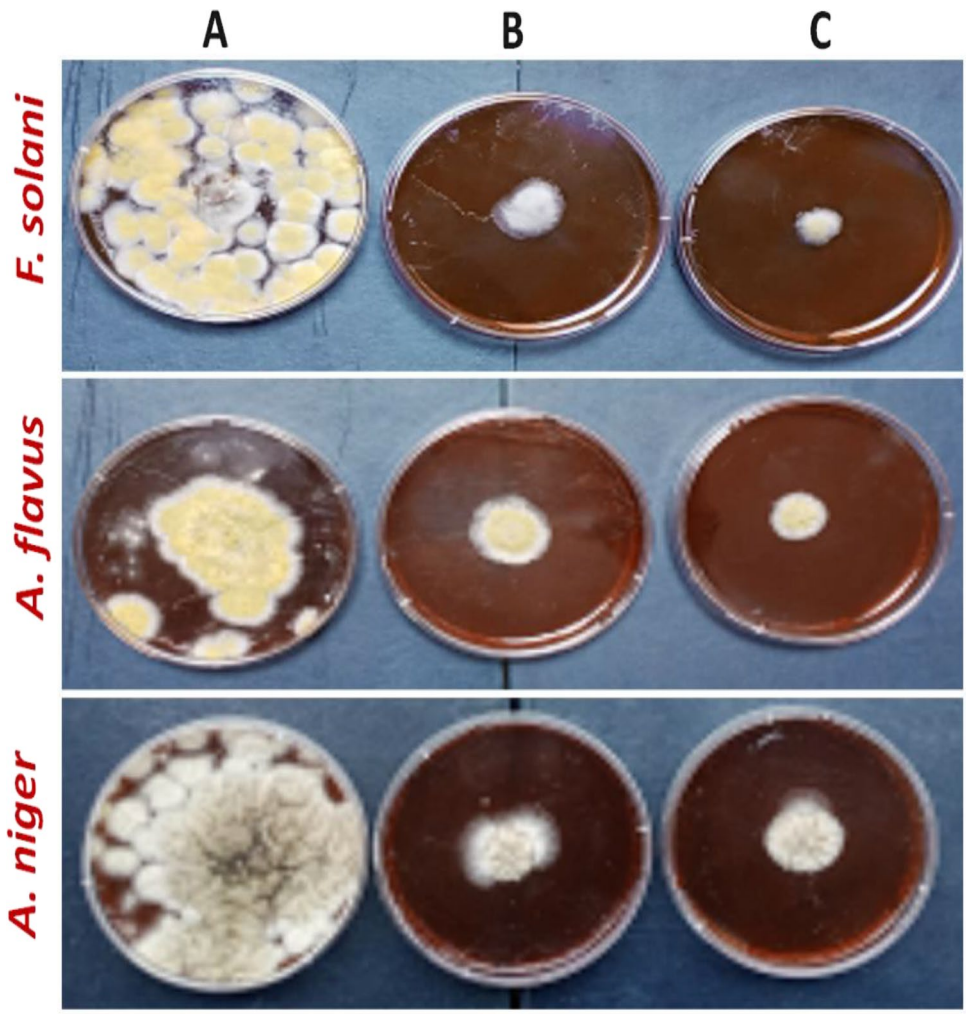


Fig. 6 Antifungal activities of biosynthesized AgNPs (150 µg/ml) from feather hydrolysates produced by *Bacillus safensis* and *Aquamicrobium defluvii*: **A** control, **B** AgNPs produced using *A. defluvii* feather hydrolysate, **C** AgNPs produced using *B. safensis* feather hydrolysate

Efficacy of FH-AgNPs for controlling fungal phytopathogens

The biogenic FH-AgNPs demonstrated excellent inhibitory activities against phytopathogenic fungal strains of *Aspergillus niger, Aspergillus flavus* and *Fusarium solani* (Fig. 6). The particles at concentration of 150 µg/ml produced over 60% fungal growth inhibition. Fungal growth inhibition induced by *B. safensis* AgNPs followed the order; *F. solani*, 88.2% > *A. niger*, 71.8% > *A. flavus*, 68.8%. In the same vein, the influence of *A. defluvii* AgNPs on the fungal pathogens was in a similar order; *F. solani*, 76.5% > *A. niger*, 69.7% > *A. flavus*, 60.33%. It was observed that the biogenic FH-AgNPs were potent against the three fungal strains with optimal effect produced against *F. solani* and *A. niger*. Nanoparticles induce antimicrobial effect by interfering with biological processes like metabolic pathways, protein synthesis and cellular structures including DNA, cell membrane and ribosome. Gautam et al. [24] reported a potent antifungal activity of biogenic AgNPs (110 µg/ml) against a phytopathogenic strain of *Fusarium* sp. Similarly, the biologically synthesized AgNPs using agrowastes significantly inhibited the growth of sugarcane fungal pathogen, *Fusarium moniliforme* [69]. In a related study, Azeez et al. [11] reported a very considerable antifungal activity of biosynthesized AgNPs against *Fusarium* spp and *Aspergillus* sp isolated from *C. olitorius,* while Mahmood et al. [69] used biofabricated AgNPs to control sugarcane phytopathogens.

Phytopathogens that include bacteria, fungi and viruses mediate diseases that pose high loss of yield and quality deterioration to agricultural foods. This and other factors like climate change contribute immensely to global food insecurity [24]. In this sense, nanotechnology as a rapidly growing modern technology has emerged as a promising alternative to the conventional use of pesticides and antibiotics for plant diseases' management, due to their unique physiochemical properties like large surface area to volume ratio, solubility, antimicrobial properties and prolonged residual activity [70]. Hence, the remarkable antifungal activities displayed by the biogenic FH-AgNPs as obtained in this finding showed its promising application

Table 7 Comparative analysis of performance of FH-AgNPs on grown vegetables

Parameters	*B. safensis* FH-AgNPs	*A. defluvii* FH-AgNPs
Shoot height (cm)	26.75 ± 1.08—38.54 ± 1.78	28.76 ± 2.50—44.78 ± 1.59
Germination rate index	15.31 ± 0.58—22.33 ± 0.81	15.44 ± 0.86—22.98 ± 0.61
Chlorophyll content	1.09 ± 0.19—1.98 ± 0.16	1.09 ± 0.09—1.85 ± 0.22
% H_2O_2 scavenged	61.89 ± 0.67—66.21 ± 0.17	59.72 ± 0.87—64.76 ± 0.51
% DPPH scavenged	44.13 ± 0.33—48.63 ± 0.28	40.85 ± 0.28—47.97 ± 0.24
% LPO inhibition	76.86 ± 0.72—79.68 ± 1.16	76.96 ± 1.02—81.01 ± 1.20
Catalase activities (unit/mg protein)	275.45 ± 0.51—309.83 ± 0.36	263.11 ± 0.17—297.13 ± 1.06
Flavonoids (mg/g of catechin)	55.74 ± 0.16—65.74 ± 1.03	59.67 ± 0.26—66.85 ± 0.37
Total phenols (mg/g of gallic acid)	245.13 ± 3.62—283.62 ± 2.67	296.13 ± 2.57—305.22 ± 3.12
Total Proanthocyanidin (mg/g of catechin)	85.33 ± 2.18—127.13 ± 1.95	119.62 ± 1.33—125.16 ± 1.82

as a biological tool for the effective and sustainable control of phytopathogenic fungi. Thus, the FH-AgNPs can suitably act as nanofertilizers and nanopesticides simultaneously, with the potential to reduce cost of inputs in crop production.

Comparative analysis of performance of FH-AgNPs on grown vegetables

Comparatively, it was observed that the FH-AgNPs of both *B. safensis* and *A. defluvii* demonstrated very similar phytostimulatory effects on the grown vegetables as there was no considerable difference in the values obtained on their growth characteristics. The FH-AgNPs of *B. safensis* and *A. defluvii* optimally enhanced the shoot height of the vegetables in the range of 26.75 ± 1.08—38.54 ± 1.78 cm and 28.76 ± 2.50—44.78 ± 1.59 cm, respectively (Table 7). In addition, the FH-AgNPs of the two bacterial strains elevated the antioxidant activities and the phytochemical compositions of the vegetables in a similar manner. However, the FH-AgNPs of *B. safensis* produced better effect on the catalase activity (309.83 ± 0.36 unit/mg protein) than FH-AgNPs of *A. defluvii* (297.13 ± 1.06 unit/mg protein), while the *A. defluvii* FH-AgNPs treatment was better in total phenols determination (305.22 ± 3.12 mg/g of gallic acid) than *B. safensis* FH-AgNPs (283.62 ± 2.67 mg/g of gallic acid). The slight disparities recorded in the performances of the FH-AgNPs produced by the two bacterial strains may be attributed to variations in the compositions of their FHs or metabolic products involved in the bioreduction of Ag^+ to produce the biogenic AgNPs [46].

Conclusion

This study has demonstrated the biosynthesis of FH-AgNPs using feather hydrolysates obtained after the degradation of chicken feather by *Bacillus safensis* LAU 13 and *Aquamicrobium defluvii* FH 20. The biogenic FH-AgNPs as nanofertilizers enhanced the seed germination, shoot height, root length, leaf sizes, chlorophyll contents and other growth parameters of *Corchorus olitorius, Amaranthus caudatus* and *Celosia argentea* up to 1.58-fold compared to controls. The AgNPs-fertilized vegetables were richer in phytochemicals including total phenols, flavonoids and total proanthocyanidin compounds and they displayed potent hydrogen peroxide and DPPH free radicals scavenging abilities better than water and NPK-fertilized plants. The FH-AgNPs improved the catalase activity of the vegetables as well as their hepatoprotective properties as they induced lipid peroxidation inhibition against ferrous ion (Fe^{2+}) damage in the precision-cut liver slices up to 1.21-fold improvement over the untreated plants. The FH-AgNPs demonstrated very remarkable inhibitory activities against phytopathogenic fungal strains of *Aspergillus niger, Aspergillus flavus* and *Fusarium solani*. The results obtained in this study indicate that the biogenic FH-AgNPs can be applied in smaller amounts in comparison with synthetic fertilizers whose excessive use often leads to toxicity in plant and huge detrimental effects in the environment. Furthermore, the biogenic FH-AgNPs not only enhanced the plant growth like some synthetic fertilizers but also inhibited the proliferation of some phytopathogens. Hence, the biogenic FH-AgNPs can be deployed as a better substitute to the conventional synthetic fertilizer and pesticides whose production and application constitute serious ecological threat for an improved, safe and sustainable agricultural food production. To the best of our knowledge, this is the first application of feather hydrolysate-mediated AgNPs in growing plants.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest Authors declare no conflict of interest.

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