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Research article

Phytomodulatory effects of silver nanoparticles on *Corchorus olitorius*: Its antiphytopathogenic and hepatoprotective potentials

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ABSTRACT

This study has reported the effects of biogenic silver nanoparticles (AgNPs) using cocoa pod extract on physiological tolerance indices, antioxidant activity and hepatoprotective potentials of *Corchorus olitorius* as well as its efficiency for controlling soil phytopathogens. *C. olitorius* seeds were grown in soil prepared with water (control), 0.05, 0.1, 0.15 and 0.2 mg AgNPs/g soil. *C. olitorus* grown with AgNPs had significantly ($p < 0.05$) higher free radical scavenging ability, ferric reducing ability, percentage germination, vigour indices, longer roots and shoots as well as lower moisture content over control. *C. olitorius* grown with AgNPs attenuated hydrogen peroxide (H_2O_2)-mediated reduction in catalase concentrations and H_2O_2-induced malondialdehyde elevations in liver. Efficiency of AgNPs to reduce soil phytopathogens (fungi and nematodes) revealed significant ($p < 0.05$) reduction in the incidences of soil and shoot *Meloidogyne* spp., *Aspergillus terreus*, *A. niger*, *Fusarium* spp. and *Cladosporium* spp. with increase in concentrations of AgNPs. More efficiently, there was complete extermination of *A. niger* and *Fusarium* spp. in the leaves of *C. olitorius* grown with AgNPs. Results in this study have shown the positive influence of AgNPs on *C. olitorius* by strengthening its resistance against fungi, and nematodes, improvement of its shelf-life, modulation of antioxidant activities and promotion of liver-detoxifying potentials.

1. Introduction

Vegetables are important components of the ecosystem and play prominent roles as sources of nutrients needed for human consumption. Their productivity and growth are controlled by soil conditions such as bioavailability of nutrients (organic, macro and micro minerals), pH, soil texture and various microbial population; some of which are beneficial being responsible for transforming organic matters and regulating nutrient cycles while others are parasitic and thus affect the quality as well as quantity of vegetables (Ashrafi et al., 2010a,b; Pallavi et al., 2016; Shalaby et al., 2016; Pérez-de-Luque, 2017; Annu et al., 2018). Quality of vegetables is determined by its health-promoting abilities resulting from nutritional and bioactive components contained in them. Bioactive components equally assist vegetables to build up resistance to soil phytopathogens through synthesis of secondary metabolites which accumulation and biotransformation improve the survival of vegetables (Azeez et al., 2017a, b; Marslin et al., 2017). This invariably contributes to increase in antioxidant activities due to improvements in phytochemical constituents (Sharma et al., 2012; Khodakovskaya et al., 2013; Azeez et al., 2017a, c; Hernández-Hernández et al., 2017).

Corchorus olitorius Linn (Jute Mallow); an edible vegetable used for preparing soup contains flavonoid and phenolic compounds with very high antioxidant activity which are vital for protection of organs against free radical injury. It possesses anti-atherosclerotic, anti-helminthic, antimicrobial, anticancer, anti-inflammatory, antidiabetic and antipyretic properties which are related to polyphenolic contents (Olajire and Azeez, 2011; Gbadamosi et al., 2012; Mahbubul, 2013). Studies have shown recently that the quality of vegetables could be improved with the introduction of nanoparticles (Sharma et al., 2012;

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Wang et al., 2013; Azeez et al., 2017c; Gupta et al., 2018). However, there is dearth of information on the applications of nanoparticles to improve growth and production of phytochemicals in *C. olitorius,* although the plant has been used for the biosynthesis of some nanoparticles (El-Rafie et al., 2016; Meva et al., 2016).

Contrasting results involving both phytotoxic and phytostimulatory effects of metal nanoparticles applications in plants have been obtained in experimental studies. Studies have shown that metal nanoparticles affected seed germination, photosynthetic pigment, plant growth, gene expression, root colouration and induced oxidative stress, DNA damage, protein damage, genotoxicity, physiological and cellular toxicities in *Arabidopsis thaliana*, *Zea mays*, tobacco seedlings and *Lolium multiflorum* (Geisler-Lee et al., 2013; Qian et al., 2013; Li et al., 2018; Saha and Dutta Gupta, 2017; Tripathi et al., 2017; Ke et al., 2018; Štefanić et al., 2018). Although, mechanisms of metal nanoparticles phytotoxicity are not well understood, they could be attributed to factors such as seed viability, seed species, temperature, duration of plant exposure to nanoparticle concentration and their application method as well as release of Ag^+ from AgNPs (Mishra and Singh, 2014; Saha and Dutta Gupta, 2017; Tripathi et al., 2017; Ke et al., 2018; Štefanić et al., 2018). Uptake and translocation of nanoparticles are affected by chemical composition, concentration, mode of synthesis (biological or chemical), size, aggregation state, bioavailability in soil, stability, shape and ratio of surface area to volume. Equally, physicochemical processes in soil can affect nanoparticles bioavailability (Raliya et al., 2015; Pallavi et al., 2016; Azeez et al., 2017c; Pérez-de-Luque, 2017; Tripathi et al., 2017). Chemical composition, mode of synthesis and release of ionic silver from AgNPs are very important factors to consider when evaluating the potentials of metal nanoparticles to improve or induce phytotoxicity in vegetables. Some studies equally posited that the toxicity arising from release of ionic silver from AgNPs was due to inhibition of activities of critical abiotic receptors by ionic silver (Yin et al., 2011; Geisler-Lee et al., 2013; Wang et al., 2013; Mishra and Singh, 2014).

Conversely, phytostimulatory effects of metal nanoparticles especially AgNPs have been well reported and are attributable to their abilities to penetrate, translocate, engineer electron exchange and develop favourable interactions with vegetables which enhance photosynthesis, water utilization, nitrogen metabolism, seed germination, cell division and elongation that are needed for vegetable growth to promote physiological activities and control plant diseases (Krishnaraj et al., 2010; Sharma et al., 2012; Raliya et al., 2015; Shalaby et al., 2016; Azeez et al., 2017c; Chaudhuri and Malodia, 2017; Hernández-Hernández et al., 2017; Yang et al., 2017; Gupta et al., 2018). Chemical composition of nanoparticles containing silver seems preferable due to distinct, stronger, broader biocidal properties of zero-valent nanosilver (Schluesener and Schluesener, 2013; Ivask et al., 2014, Lateef et al., 2016a, b; Zuverza-Mena et al., 2017). Moreover, AgNPs influence enlargement of root pores that aids better nutrients uptake and translocation of such through epidermis, cortex, endodermis and xylem to leaves (Geisler-Lee et al., 2013, 2014; Li et al., 2016; Zuverza-Mena et al., 2017). It is well established in many reports that AgNPs stimulate growth, enhance seed germination, improve antioxidant activity and can regulate soil phytopathogens due to their antimicrobial, antifungal and larvicidal activities (Sharma et al., 2012; Castiglione et al., 2016; Lateef et al., 2016c, d; Azeez et al., 2017c; Hernández-Hernández et al., 2017; Mishra et al., 2017). Biologically synthesized nanoparticles are less toxic and eco-friendlier compared with chemically synthesized particles because the capping and stabilizing agents are biological macromolecules unlike in chemical synthesis where synthetic chemicals are involved (Adelere and Lateef, 2016; Lateef et al., 2016a, b, c, d, e; Azeez et al., 2017c; Mishra and Singh, 2014; Annu et al., 2018). Furthermore, previous studies have reported that toxicity of nanoparticles is connected to its mode of synthesis, size, concentration and bioaccumulation in the environment (Syu et al., 2014; Adelere and Lateef, 2016; Gupta et al., 2018).

Soil phytopathogens have devastating effects on quality and quantity of marketable yields of vegetables. Many control measures such as the use of conventional fungicides have been applied but their environmental polluting effects, high cost and toxicity are issues of concern. Control with AgNPs provides an alternative to regulate, and render soil phytopathogens ineffective towards vegetables due of their better efficacy, stability against sedimentation and relative advantages compared to conventional biopesticides as well as its ability to promote other factors that directly stimulate or strengthen vegetable productivity in addition to physiological tolerance (Jo et al., 2009; Khot et al., 2012; Ali et al., 2017; Mishra and Singh, 2014). AgNPs due to their wide spectrum of antimicrobial properties have gained prominence as potent antiphytopathogenic agents that could kill approximately 650 species of plant pathogens (Shahrokh and Emtiazi, 2009). It has been reported that addition of nanoparticles to soil can inhibit the growth of soil microflora and kill numerous bacterial and fungal phytopathogens such as *Fusarium culmorum*, *Colletotrichum* spp., *Pseudomonas syringae* pv, *Rhizoctonia solani* and *Sclerotium rolfsii* (Kasprowicz et al., 2010; Lamsal et al., 2011; Chu et al., 2012; Ali et al., 2017; Gupta et al., 2018). The inhibition of soil pathogen involves a complex mechanism which is associated with fungicidal and nematocidal properties of AgNPs, whereby nanoparticles can permeate the cells and interfere with cellular mechanisms through reactions with DNA and proteins (Ashrafi et al., 2010a; Lateef et al., 2016a, b, c, Pallavi et al., 2016). However, the fate of *C. olitorius* grown with AgNPs for human consumption in terms of toxicity arising from AgNPs application needs to be established, as well as its ability to mediate and protect against liver damage caused by free radicals. Thus, this study was aimed at determining the influence of biogenic AgNPs synthesized using pod extract of cocoa in stimulating growth parameters of *C. olitorius*, modulating its antioxidant activity, decimating soil phytopathogens and attenuating liver damage induced by hydrogen peroxide. To the best of our knowledge, studies have not been reported on the use of nanoparticles to modulate antioxidant activity of plant for hepatoprotection of liver injury induced by free radicals. Therefore, this study presents a novel application to achieving these objectives.

2. Materials and methods

2.1. Biosynthesis of AgNPs using cocoa pod extract

AgNPs used in this study were biologically synthesized using the pod extract of cocoa as previously reported by Lateef et al. (2016a). The scheme of the synthesis is shown in Fig. 1. The biosynthesized AgNPs were characterized by UV–Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM) following standard procedures (Lateef et al., 2016a). The concentration of synthesized AgNPs was determined by concentrating a known volume of colloidal AgNPs through high speed centrifugation (10, 000 rpm) and drying at 60 °C followed by weight determination on Metler balance. The concentration of stock AgNPs synthesized was 100 mg/l.

2.2. Soil sampling and planting of C. olitorius seeds

Soil samples were collected from vegetable farm (7°76‘19.4″N and 4°60‘3.18″E) in Osun State University near botanical garden at a depth between 0 and 25 cm. Soil sample was slightly acidic (pH - 5.8), dark coloured and fertile having organic matter (14.8 $mgkg^{-1}$), nitrogen (312.2 $mgkg^{-1}$), phosphorus (14.3 $mgkg^{-1}$) determined using method of Page et al. (1982). It composed of Fe^{2+} (0.028 $mgkg^{-1}$ soil), K^+ (2501.1 $mgkg^{-1}$ soil), Na^+ (110.85 $mgkg^{-1}$ soil), Ca^{2+} (31.6 $mgkg^{-1}$ soil), Mg^{2+} (2361.17 $mgkg^{-1}$ soil) determined using microwave plasma coupled with atomic emission spectrometer (MP-AES – model MY14280004) after digestion of soil sample with concentrated HNO_3 and HCl (7:3).

C. olitorius seeds were procured from a seed vending shop at Oja-Oba market in Osogbo, Osun State Nigeria, soaked in warm water

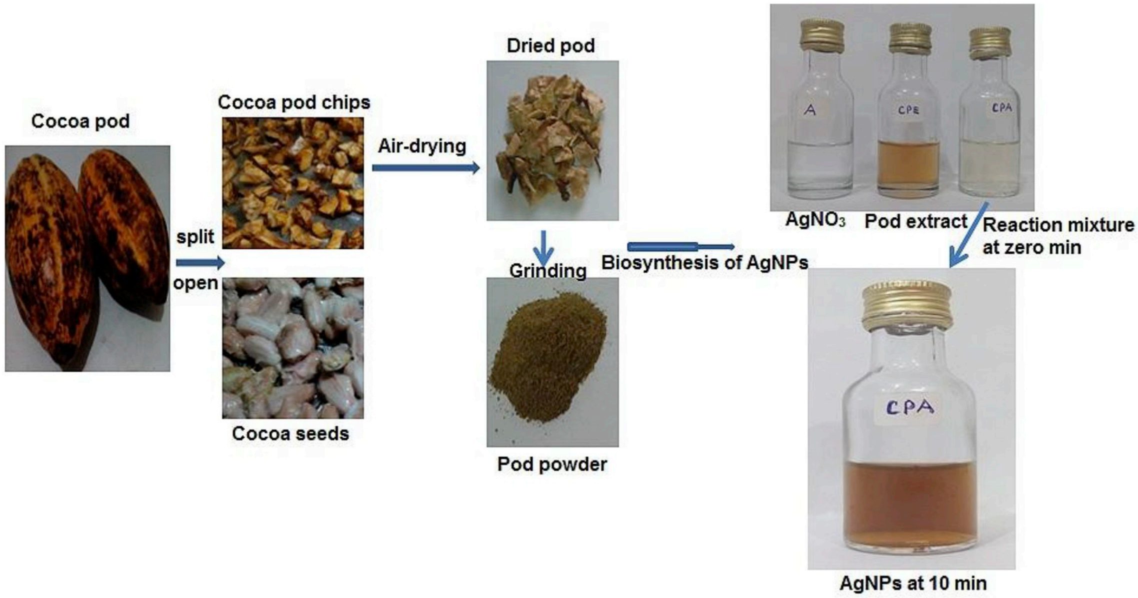
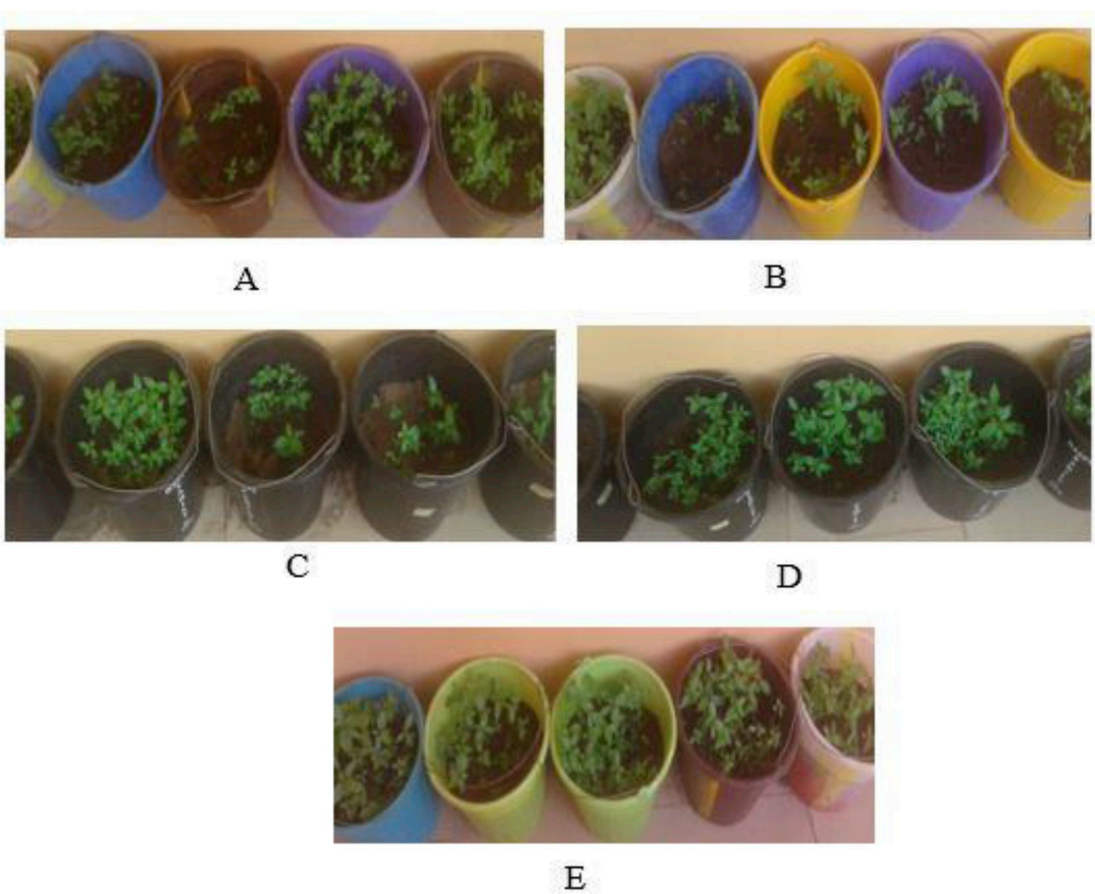


Fig. 1. Biogenic synthesis of AgNPs from cocoa pod (Lateef et al., 2016a).



A: Control: *C. olitorus* grown without AgNPs, **B**: *C. olitorus* grown in 0.05 mg AgNPs/g soil, **C**: *C. olitorus* grown in 0.1 mg AgNPs/g soil, **D**: *C. olitorus* grown in 0.15 mg AgNPs/g soil **E**: *C. olitorus* grown in 0.2 mg AgNPs/g soil

Fig. 2. Effects of various concentrations of AgNPs on *C. olitorus* growth.

overnight so as to soften the seed coats. Twenty-five 7-L capacity buckets were half-filled each with 250 g of 2 mm wire-mesh filtered soil of density 0.07 g/ml (five for each group). Five buckets for each group were soaked with 100 ml of water (control), 25, 50, 75 and 100 mg/l AgNPs solutions to achieve initial concentrations of 0 (control), 0.01, 0.02, 0.03 and 0.04 mg AgNPs/g soil. These groups were designated as A, B, C, D and E. This was followed by sowing six (6) seeds in each bucket, to give a total of thirty (30) seeds per group. The seeds were watered for four weeks with AgNPs solutions for each group to achieve final concentrations of 0 (control), 0.05, 0.1, 0.15, 0.2 mg AgNPs/g soil (Fig. 2).

Planting experiments were conducted at temperature (27.55 ± 3.45 °C), relative humidity (47.23 ± 2.83%), UV index (5.55 ± 0.15) and light intensity (10290 illuminance).

2.3. Determination of growth parameters and moisture content of C. olitorius

C. olitorius were harvested after 4 weeks of planting and different growth parameters were determined. Germination percentage was determined using equation (1). Root and shoot lengths were measured using a metric rule. Vigour index was calculated using equation (2) while the moisture content was determined as reported by Azeez et al. (2012).

$$\% \; Germination = \left(\frac{number\ of\ germinated\ seeds}{total\ number\ of\ seeds} \right) \times 100 \quad (1)$$

$$Vigour\ index = (root\ length + shoot\ length) \times \%\ germination \quad (2)$$

2.4. Control of soil phytopathogens: fungi and nematodes

Soil samples from each bucket were collected up to a depth of 8 cm from the upper surface and Potato dextrose agar (PDA) was prepared for the isolation of fungi. Soil, *C. olitorius* shoot and leaf solutions (both treated with and without AgNPs) were prepared aseptically by introducing 1 g of each into 10 ml of distilled water and then serially diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Then, 1 ml each from the selected tube was introduced to PDA agar which was incubated at 25 °C for seven days for the growth of fungi. Isolates obtained were purified to obtain pure cultures, identified and characterized based on their colonial and morphological characteristics. The microscopic features of fungi were compared with fungal compendium (Domsch et al., 1980). Fungal isolates were identified and characterized up to the genus and species level presumptively. Identification was done on the basis of morphological and colonial features using the procedures of Guy and Richard (2001).

Furthermore, nematodes were extracted from soil and roots of *C. olitorius* grown with and without AgNPs using Cobb sieving and gravity methods as described by Siddiqi and Booth (1991). The recovered nematodes were examined and counted using the compound microscope. Morphological identification was done using perineal patterns for species identification as described by Fortuner (1988).

2.5. Extraction and determination of antioxidant activities of C. olitorius

Two gram of *C. olitorius* sample was extracted twice with 150 ml of 70% aqueous methanol, filtered and concentrated. Absorbance of free radical scavenging ability of each extract and control using 2,2-diphenyl-1- picrylhydrazyl (DPPH) was read at 517 nm according to the method reported by Azeez et al. (2017a), while the ability of each extract to reduce Fe^{3+} to Fe^{2+} as ferric reducing activity was done using the procedure described by Lateef et al. (2016b).

2.6. Experimental animals

Two male albino rats (*Rattus norvegicus*) of Wistar strain weighing 100–120 g were purchased from the Department of Physiology, College of Health Sciences, Osun State University, Osogbo, Nigeria. The animals were handled according to the NIH guidelines for the care and use of laboratory animals (NIH, 1985) in accordance with the principles of Good Laboratory Procedure (GLP) (WHO, 1998).

They were housed within the Animal House of the Department of Biochemistry, Faculty of Basic and Applied Sciences, Osun State University at room temperature, and maintained under a 12 h light/12 h dark cycle, with feeds and water available *ad libitum*. The animals were allowed to acclimatize for six days prior to sacrifice.

2.6.1. Preparation of precision-cut liver slices

The procedure described by Haniya and Padma (2013) was used to prepare the precision-cut liver slices (PCLS), with slight modification. Twenty-four hours post-acclimatization, the animals were sacrificed by cervical dislocation. A midline abdominal incision was made to open up the abdominal cavity and access the visceral organs. The liver was excised and quickly placed in ice-cold phosphate buffered saline (PBS; pH 7.4). Precision-cut liver slices (PCLS; 0.25 g; 2 mm thick) were thereafter prepared and placed in ice-cold PBS (pH 7.4). Six treatment groups were set up, each in four replicates (1 ml final volume). Group I (control) contained PCLS and PBS only, while groups II − VI contained PCLS, 500 μM H_2O_2 and 0.2 ml of extracts of *C. olitorius* grown without biogenic AgNPs and *C. olitorius* grown in 0.05, 0.1, 0.15, 0.2 mg AgNPs/g soil respectively, in Eppendorf tubes. All tubes were incubated at 37 °C for 1 h with intermittent shaking. The PCLS mixtures were thereafter homogenized and centrifuged for 15 min at 1500 rpm to obtain clear supernatants. The control and treatment groups were carefully and sufficiently chosen to investigate hepatoprotective potentials of *C. olitorius* grown with and without AgNPs on liver cells induced H_2O_2 damage.

2.6.2. Determination of ex vivo catalase activity and lipid peroxidation product

The determination of *ex-vivo* catalase activity and malondialdehyde (MDA) concentrations were carried out using standard procedures for MDA (Buege and Aust, 1978) and catalase (Beers and Sizer, 1952).

MDA in liver supernatants was determined spectrophotometrically by evaluating its reaction with thiobarbituric acid (TBA) forming a 1:2 adduct (MDA-TBA2) which produces a complex aromatic structure. Briefly, 0.1 ml of the liver supernatants was added to 2 ml of TBA-TCA-HCl reagent in the ratio of 1:1:1 (0.3% TBA, 0.25 N HCl and 15% trichloroacetic acid, TCA). The mixture was then placed in boiling water bath for 15 min, cooled and centrifuged at 550 rpm for 30 min. The absorbance of the clear supernatant was read at 535 nm against distilled water blank. The values were expressed as nmol MDA/mg protein using the following expression:

$$MDA = \frac{Absorbance\ x\ sample\ volume\ x\ dilution\ factor}{1.56\ x\ 10^5\ x\ total\ volume\ x\ (mg\ protein/ml)} \quad (3)$$

Catalase activity, on the other hand, was assayed based on its ability to reduce hydrogen peroxide to water and oxygen. Briefly, the reaction mixture (3 ml, final volume) contained 0.1 ml of liver supernatants and 2.9 ml of 0.036% H_2O_2. This was mixed by inversion and the time taken (min) for the absorbance to decrease from 0.45 to 0.40 units at 240 nm was recorded. Potassium phosphate buffer (3 ml, 50 mM, pH 7.0) was used as blank. The activity of catalase was determined using the expression:

$$Catalase\ activity\ (nmol/min/mg\ protein) = \frac{3.45\ x\ 1000\ x\ dilution\ factor}{minutes\ x\ volume\ x\ (mg\ protein/ml)} \quad (4)$$

Where 3.45 was the concentration of H_2O_2 (3.45 μmoles) in 3 ml of the reaction mixture.

2.7. Statistical analysis

Results of growth parameters, moisture contents, antioxidant activities, MDA and catalase are expressed as mean ± standard deviation of three replicates subjected to one-way ANOVA followed by Duncan's multiple range test (DMRT) for comparison of mean at 95% confidence level using SPSS 17 version.

3. Results and discussion

3.1. Biosynthesis of AgNPs and its effects on growth parameters and moisture contents of C. olitorius

The pod extract of cocoa facilitated the formation of brown colloidal AgNPs, which absorbed maximally at 428.5 nm. The synthesized particles were fairly spherical with average size of 15.5 ± 4.2 (Lateef et al., 2016a). The beans of cocoa have also been used for the green synthesis of AgNPs with potent biological properties (Azeez et al., 2017c).

Germination was observed on the fifth day for all the treatments. However, *C. olitorius* grown in 0.05 and 0.1 mg AgNPs/g soil had significantly ($p < 0.05$) reduced percentage germination (57.24 ± 1.81 and 61.15 ± 3.45 respectively) compared to control (72.18 ± 3.63), while *C. olitorius* grown in 0.15 and 0.2 mg AgNPs/g soil had significantly ($p < 0.05$) higher germination percentages (88.47 ± 4.87 and 92.72 ± 2.44) as shown in Fig. 3. The trend in growth rate was inverse dose-dependent which could be due to the inability of lower concentrations to accelerate parameters needed for *C. olitorius* growth. This is in consonance with the report of Wang et al. (2017). Growth parameters are used to assess enhancement and toxicity of nanoparticle application in plant production (Praveen et al., 2018). They are also used as measures of ability of seeds to develop into plant. The trend in this study (inverse dose-dependent) could be ascribed to the influence of higher concentrations of biogenic AgNPs to enhance the activities of enzymes needed for improved seed germination and seedling growth (Wang et al., 2017). Furthermore, AgNPs trigger complex physiological processes involving alteration of expression of genes that are involved in multiple cellular pathways, including cell proliferation, photosynthesis and hormone signalling pathways, including auxin, abscisic acid and ethylene (Syu et al., 2014). These results are in consonance with reports of Arora et al. (2012), Sharma et al. (2012), Khodakovskaya et al. (2013), Jayarambabu et al. (2015) and Praveen et al. (2018) on studies using AgNPs, carbon nanotubes, nano-ZnO, and Fe_3O_4 nanoparticles as positive regulators of plant growth.

Root and shoot became longer with increase in concentration of AgNPs in soil for *C. olitorius* ranging from 2.63 to 3.62 cm for roots and 7.93–9.65 cm for shoots. Though, no significant ($p > 0.05$) increase was obtained between *C. olitorius* grown on 0.05, 0.1 mg AgNPs/g soil and control, however, *C. olitorius* grown on 0.15 and 0.2 mg AgNPs/g soil had significantly ($p < 0.05$) longer roots and shoots as shown in Fig. 3. AgNPs-mediated increase in root and shoot lengths might be connected to the ability of AgNPs to promote plant regulators required for cell divisions, cell elongation as well as enlargement of root pores which enhance nutrients and mineral uptake (Lu et al., 2002; Shah and Belozerova, 2009; Miralles et al., 2012; Sharma et al., 2012; Raliya et al., 2015; Chaudhuri and Malodia, 2017; Hernández-Hernández, 2017). Moreover, AgNPs trigger complex physiological processes involving alteration of expression of genes that are involved in multiple cellular pathways, including cell proliferation, photosynthesis and hormone signalling pathways, including auxin, abscisic acid and ethylene (Syu et al., 2014). This is an indication that at higher concentrations of AgNPs, roots of *C. olitorius* could absorb more nutrients, minerals and enhance antioxidant enzymes activities (Zheng et al., 2005; Yasur and Rani, 2013; Geisler-Lee et al., 2014; Mirzajani et al., 2014; Syu et al., 2014; Li et al., 2016; Tripathi et al., 2017; Wang et al., 2016; Wang et al., 2017; Zuverza-Mena et al., 2017; Gupta et al., 2018).

Moisture contents significantly ($p < 0.05$) reduced with increase in concentrations of AgNPs with highest and lowest values of 87.26 and 58.75% obtained for the control and *C. olitorius* treated with 0.2 mg AgNPs/g soil respectively. This reduction was concentration-dependent and points to the fact that the biogenicAgNPs promoted water distribution efficiency in *C. olitorius* and lowered excessive water accumulation which could aid microbial degradation leading to rottenness that affects both its quality (nutritional and bioactive components) and quantity (Lu et al., 2002; Azeez et al., 2012; Azeez et al., 2017a).

Vigour indices of *C. olitorius* grown in water, 0.05, 0.1, 0.15, 0.2 mg AgNPs/g soil significantly ($p < 0.05$) followed the trend; 0.05 mg AgNPs/g soil < 0.1 mg AgNPs/g soil < control < 0.15 mg AgNPs/g soil < 0.2 mg AgNPs/g soil as shown in Fig. 4. Vigour index presents the influence of additive effects of various growth regulators on *C. olitorius* and how physiologically tolerant the seeds were to these factors. An increase in vigour index is an indicator of how AgNPs boosted seed viability, quality and growth profile of *C. olitorius* which could have resulted from enhancement of its physiological activities (Sharma et al., 2012; Prasad et al., 2012; Shaheb et al., 2016; Wang et al., 2016; Cvjetko et al., 2017; Tripathi et al., 2017).

3.2. Efficiency of AgNPs for controlling soil phytopathogens on C. olitorius

3.2.1. Isolation of fungi and nematodes from soil and C. olitorius

The fungi isolated from control, AgNPs-enriched soil, AgNPs grown *C. olitorius* shoots and leaves include strains of *Aspergillus flavus, A.*

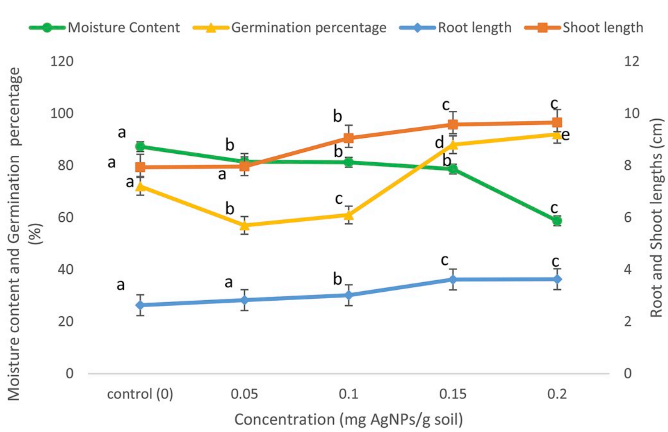


Fig. 3. Moisture contents, percentage germination, root and shoot lengths of *C. olitorus* grown in water and 0.05, 0.1, 0.15, 0.2 mg AgNPs/g soil. Data points with different superscripts are significantly different ($p < 0.05$).

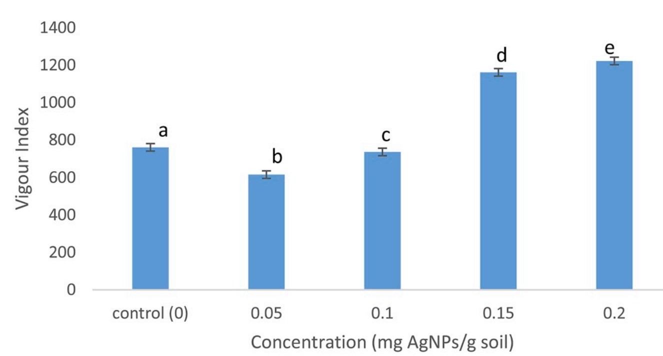


Fig. 4. Vigour index of *C. olitorus* grown in water and 0.05, 0.1, 0.15, 0.2 mg AgNPs/g soil. Data points with different superscripts are significantly different ($p < 0.05$).

Table 1
Fungi isolated and their occurrence in soil, shoot and leave samples of *C. olitorus* grown with and without AgNPs.

	Concentration (mg AgNPs/g soil)														
	Control (0)	0.05	0.1	0.15	0.2	Control (0)	0.05	0.1	0.15	0.2	Control (0)	0.05	0.1	0.15	0.2
Fungi	Soil					Shoot					Leaf				
Aspergillus niger	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Aspergillus flavus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus terreus	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhizopus sp.	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Fusarium sp.	+	+	+	+	+	+	-	+	-	-	+	+	-	+	-
Penicillium sp.	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Cladosporium sp.	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Phialophora sp.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

niger, A. terreus, Rhizopus spp., *Fusarium* spp., *Penicillium* spp., *Cladosporium* spp. And *Phialophora* spp (Table 1). The major species of fungi isolated from *C. olitorius* shoots and leaves were *A. flavus, Fusarium* spp. and *A. niger*. Frequency of occurrence of fungi decreased in shoots and leaves of *C. olitorius* grown with AgNPs, while complete reduction of some fungi were recorded suggesting that AgNPs inhibited the uptake of some fungi. It is fully established in the literature that *Fusarium* spp. has pathogenic effects on a variety of crop plants. In this study, our results show that AgNPs was effective against *Fusarium* spp. and *Aspergillus niger*. These two fungi are known plant pathogens and decrease in their prevalence is indicative of antifungal properties of AgNPs (Wharton et al., 2006; Ashrafi et al., 2010a, b; Khodakovskaya et al., 2013; Lateef et al., 2016a, b, c; Pallavi et al., 2016; Azeez et al., 2017; Mishra et al., 2017). Our findings are in agreement with well-established reports that AgNPs possesses antiphytopathogenic activities stemming from its antimicrobial properties against plant pathogens such as *Fusarium culmorum*, *Colletotrichum* spp., *Pseudomonas syringae* pv, *Rhizoctonia solani* and *Sclerotium rolfsii* (Kasprowicz et al., 2010; Lamsal et al., 2011; Chu et al., 2012; Mishra and Singh, 2014; Ali et al., 2017; Gupta et al., 2018).

Meloidogyne spp. is the highest-occurring nematode in the soil. Its population and effects on root weight and leaves of *C. olitorius* are presented in Table 2. The results indicate that population of *Meloidogyne* spp. followed the order; control > 0.05 mg AgNPs/g soil > 0.1 mg AgNPs/g soil > 0.2 mg AgNPs/g soil > 0.15 mg AgNPs/g soil.

The influence of AgNPs treatment on root weight as affected by nematode followed control > 0.05 mg AgNPs/g soil > 0.1 mg AgNPs/g soil > 0.15 mg AgNPs/g soil > 0.2 mg AgNPs/g soil, while leaf diameter followed the trend control = 0.05 mg AgNPs/g soil > 0.1 mg AgNPs/g soil > 0.2 mg AgNPs/g soil > 0.15 mg AgNPs/g soil.

The study revealed that population of *Meloidogyne* spp. exhibited great variability at all concentration levels. The decrease in the population of *Meloidogyne* spp. could be due to marked degeneration of *Meloidogyne* spp. vermiforms in the soil resulting from AgNPs application. However, the sudden rise in the population of *Meloidogyne* spp. in 0.2 mg AgNPs/g soil could be attributed to the evasive ability of nematodes to shed the components of the cuticle (Kover and Schaal, 2002).

Table 2
Meloidogyne spp population in soil and its effects on root and leaves of *C. olitorius* grown with and without AgNPs.

Concentration (mg AgNPs/g soil)	*Meloidogyne* population	Mean root weight (g)	Mean leaf produced (d)
0 (control)	920	5.26	0.65
0.05	638	5.25	0.65
0.1	216	5.17	0.70
0.15	3	5.05	0.75
0.2	36	5.0	0.72

d – diameter, g - gram.

Meloidogyne spp. infestation of *C. olitorius* leaves had an inhibitory influence on root weight and leaf diameter of control when compared with AgNPs-grown *C. olitorius*. This could have contributed to the reduced growth profile obtained for *C. olitorius* grown with water. However, this contradicts the findings of Imafidor and Nzeakor (2008) whose work suggested that the presence of *M. javanica* probably stimulated increase in the shoot weight of tomatoes. This study shows that *Meloidogyne* spp. inhibited the growth of shoot, height and girth of jute plant studied. Although, this work did not identify the nematode to the species level, further work needs to be done to determine the effect of *M. javanica* on the growth of shoot, height and girth of jute plant leaves.

3.3. Antioxidant activity of C. olitorius grown with AgNPs

The results of free radical scavenging and ferric reducing capacities of *C. olitorius* are shown in Fig. 5 with significant improvement ($p < 0.05$) along with increase in concentrations of AgNPs in soil. The highest concentration (0.2 mg AgNPs/g soil) and control (water) had the highest (81.37%) and lowest (45.09%) antioxidant activities as well as ferric reducing capacities (highest: 51.1%) and (lowest: 10.34%) respectively in *C. olitorius*. The potential of *C. olitorius* to reduce Fe^{3+} to Fe^{2+} is in consonance with its ability to scavenge free radicals which is concentration-dependent. Increase in antioxidant activity is ascribed to the activation of plant antioxidant defence system through alteration in secondary metabolism to consume free radicals generated by AgNPs when it was absorbed by *C. olitorius* (Sharma et al., 2012; Azeez et al., 2017c; Cvjetko et al., 2017; Tripathi et al., 2017). Equally, it indicates that AgNPs has the ability to modulate redox status of Fe^{3+} to $Fe^{2+}$and improve electron exchange efficiency in *C. olitorius* (Sharma et al., 2012; Lateef et al., 2016a, b; Hernández-Hernández et al., 2017; Pérez-de-Luque, 2017). Ferric ion reducing antioxidant assay provides an

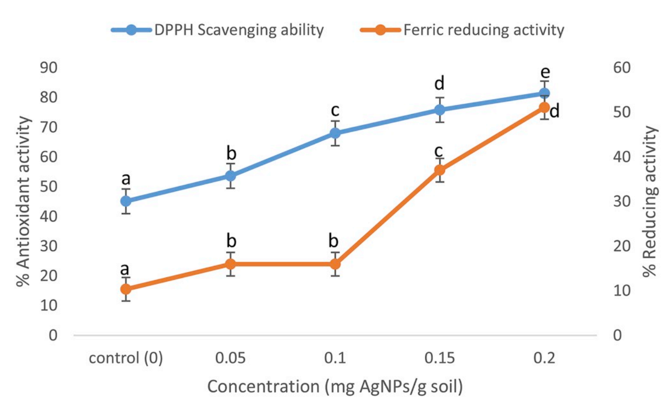


Fig. 5. Antioxidant and ferric reducing activities of *C. olitorus* grown in and 0.05, 0.1, 0.15, 0.2 mg AgNPs/g soil. Data points with different superscripts are significantly different ($p < 0.05$).

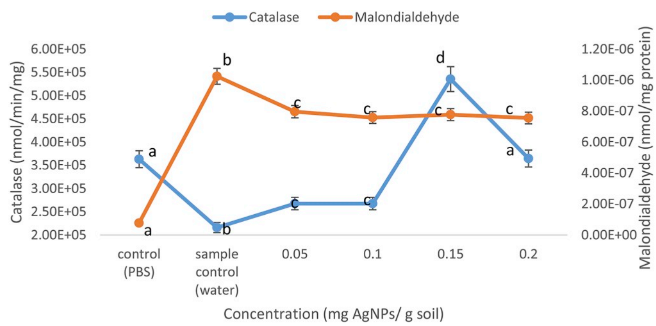


Fig. 6. Catalase concentration and lipid peroxidation levels in liver homogenates untreated and treated with *C. olitorus* grown in 0.05, 0.1, 0.15, 0.2 mg AgNPs/g soil. Data points with different superscripts are significantly different ($p < 0.05$).

index to assess reducing potentials of bioactive compounds in biological materials against oxidative stress. Equally, Fe^{3+} has low solubility, more stable and is less readily bioavailable thus needed to be converted to Fe^{2+} (Ilbert and Bonnefoy, 2013). More so, strong correlation between antioxidant activity and phytochemical content of a vegetable, *Amaranthus caudatus* grown with biogenic AgNPs has been previously reported (Azeez et al., 2017c).

3.4. *Ex-vivo lipid peroxidation and catalase activity*

This study used PCLS as a model which combines both *in-vitro* and *in-vivo* systems and has been used to study drug metabolism and predict drug toxicity in hepatocytes (Yaidikar and Thakur, 2014; Wang et al., 2017). Hydrogen peroxide was used as an oxidant which can be converted to hydroxyl radicals that initiate lipid peroxidation and cause damage to cellular macromolecules such as DNA (Chanda and Dave, 2009) by suppressing the cellular antioxidant system.

There was significantly ($p < 0.05$) elevated malondialdehyde (MDA) concentration in the PCLS treated with AgNPs-free *C. olitorius* extract (group II) when compared with the control. However, the extract of *C. olitorius* grown in 0.05, 0.1, 0.15, 0.2 mg AgNPs/g soil significantly ($p < 0.05$) attenuated H_2O_2-induced MDA elevation to the tune of 87.9% (Fig. 6). This attenuation was not concentration-dependent and, though significant, was not total because the values were still significantly ($p < 0.05$) higher than the control value.

MDA is a product of lipid peroxidation and its concentration is a biomarker of cellular oxidative stress (Karabulut-Bulan et al., 2008). The ability of *C. olitorius* grown in biogenic AgNPs soaked soil to attenuate the H_2O_2-induced MDA elevation is an indication of its *in-vivo* antioxidant activity and activation of xenobiotics metabolism enzymes such as glutathione peroxidase resulting from application of AgNPs (Ajiboye et al., 2010; Sharma et al., 2012; Oloyede et al., 2013; Hernández-Hernández et al., 2017; Rizwan et al., 2017).

Hydrogen peroxide mediated a significant ($p < 0.05$) reduction in catalase activity which was significantly ($p < 0.05$) attenuated by *C. olitorius* grown in 0.05, 0.1,0.15, 0.2 mg AgNPs/g soil. This attenuation was also not concentration-dependent with the *C. olitorius* grown in 0.15 and 0.2 mg AgNPs/g soil producing catalase activities higher than the control value, and the 0.05 and 0.1 mg AgNPs/g soil producing the least increase in catalase activity (Fig. 6).

Catalase is a primary antioxidant enzyme involved in direct elimination of oxidants by reduction of H_2O_2 to water and oxygen, and its activity has been used as a marker of *in-vivo* antioxidant capacity (Liou and Storz, 2010; Yang et al., 2017). The ability of *C. olitorius* grown with biogenic AgNPs to reverse H_2O_2-mediated reduction in catalase activity supports the potential antioxidant capacity of the extracts. This is an indicator of increased catalase activity in *C. olitorius* grown with AgNPs generated in defense of *C. olitorius* when AgNPs was absorbed by the roots (Juárez-Maldonado et al., 2016; Cvjetko et al., 2017; Pinedo-Guerrero et al., 2017; Tripathi et al., 2017). This could come from plants *in-vivo* antioxidant activities which have ability to reduce lipid peroxidation and enhance activities of enzymic antioxidants as reported by Salau et al. (2015, 2016).

4. Conclusion

Results in this study have shown that biogenic AgNPs could be used to enhance *C. olitorius* growth, root and shoot elongation for more nutrient absorption. It could also be used to extend its shelf-life and strengthen its physiological tolerance against diseases as shown by results of moisture contents and vigour indices. AgNPs modulated biochemical properties in *C. olitorius* by improving the antioxidant ability and ferric reducing activity. *C. olitorius* grown in AgNPs soaked soil attenuated free radical damage on liver and decimated fungal and nematode population.

CRediT authorship contribution statement

Luqmon Azeez: Conceptualization. **Muhammed A. Rufai:** Formal analysis.

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