J. Bio-Sci. 32(2): 31-44, 2024 **ISSN 1023-8654**
http://www.banglajol.info/index.php/JBS/index
DOI:https://doi.org/10.3329/jbs.v32i2.75755

CHARACTERIZATION OF SILVER NANOPARTICLES SYNTHESIZED WITH FUNGI ISOLATED FROM COCOA AND ORANGE PLANTATION SOILS

OA Ogunsakin[1], EA Ekundayo[2]* FO Ekundayo[1] and OA Olalemi[1]

[1]Department of Microbiology, School of Life Sciences, Federal University of Technology, Akure, Ondo-State, Nigeria

[2]Department of Microbiology, Faculty of Sciences, University of Medical Sciences, Ondo City, Ondo-State, Nigeria

Abstract

Green synthesis of nanoparticles with various metals is being interested among researchers globally attention for due to their safety, environmental friendliness and non-toxicity in contrast to chemical synthesis. Here, fungi were isolated from rhizosphere of cocoa and orange obtained from Federal University of Technology, Akure using standard mycological techniques with the aim of synthesizing silver nanoparticles. The silver nanoparticles synthesized by these fungal isolates were characterized using Ultraviolet-Visible (UV-Vis) and Fourier Transmission Infra- Red (FTIR) spectroscopy. Seven fungi isolated from the soil samples viz. *Nigrospora sphaerica, Penicillium discolor, P. digitatum, Neurospora crassa, Alternarium infectoria, Trichophyton verrucosum* and *Mucor mucedo*. The colour transformation from yellow to brown indicated the formation of silver nanoparticles. All the isolates showed an intense peak in the wavelength range of 410-440 nm. The FTIR analysis of silver nanoparticles synthesized by *N. sphaerica, P. discolor, P. digitatum, N. crassa, A. infectoria, T. verrucosum* and *M. mucedo* revealed the existence of a band at different stretches of bonds at different peaks of 3314.36 cm^{-1}, 3310.47 cm^{-1}, 2412.22 cm^{-1}, 3912.33 cm^{-1} 3614.52 cm^{-1}, 3852.11 cm^{-1} and 2112.11 cm^{-1}, respectively. The silver nanoparticles synthesized could have the potential application in agricultural, pharmaceutical and food industries as well as petrochemical industries for the clean - up of crude oil contaminated soils.

***Key words**:* Characterization, Fungi, Green synthesis, Non-toxicity, Silver nanoparticles, Soils.

Introduction

Nanoparticles have been defined as objects or class of materials ranging in size from 1-100 nm that due to their size may differ from the bulk material (Saba 2015, Achmad et al. 2017). Silver nanoparticles (AgNPs) can be synthesized by several approaches including physical, chemical, and biological. The physical and chemical approaches of synthesizing AgNPs are often extremely expensive and non-environmental friendly due to the use of toxic, combustible, and hazardous chemicals, which may pose potential environmental and biological risk and high energy requirement (Awwad et al. 2013).

Biosynthesis of metal nanoparticles has received attention owing to their physicochemical properties and wide applications in biotechnology (Slavin et al. 2017, Goutam et al. 2020). Nanoparticles are biosynthesized when the microorganisms grab target ions from their environment and then turn the metal ions into the element metal through enzymes generated by the cell activities (Keat et al. 2015). Biosynthesized AgNPs from microorganisms such as bacteria, actinomycetes, fungi and algae is a green and eco-friendly

*Author for correspondence: eekundayo@unimed.edu.ng

technology and have been used in a variety of applications including enhancement of reaction rates (Xiangqian et al. 2011).

Filamentous fungi have been used as a potential source in the nanoparticles synthesis because their mycelia have a high surface area, which secretes large amounts of proteins that can participate directly in the production of nanoparticles Mohanpuri et al. 2008). Filamentous fungi are also often considered in biogenic production of nanoparticles due to their ability to sporulate fast and their ease of maintenance. They also likewise secrete proteins, enzymes and metabolites (Fouda et al. 2018, Spagnoletti et al. 2019). Also, fungi are better and preferred as resource for synthesis of nanoparticles to bacteria because they produced metabolites (Singh et al. 2016). Fungal strains such as *Fusarium oxysporum*, *Aspergillus fumigatus*, *A. niger*, *F. semitectum, Penicillium brevicompactum*, *Cladosporium cladosporioides* and *A. clavatus* have been previously described by Mohamed et al. (2014) to synthesize silver nanoparticles (AgNPs) extracellularly. Several studies have shown the capability of fungi obtained from soil samples to silver nanoparticles. However, there is little or no information regarding the ability of fungi from cocoa and plantain soils from Federal University of Technology, Akure to synthesize silver nanoparticles. This study was designed to close this gap.

Materials and Methods

Methods

Collection of Soil Samples

Soil samples were collected at about 10 cm depth from root areas (Rhizosphere) of cocoa and orange plantation trees located at Obanla, Federal University of Technology, Akure (FUTA) Ondo State with the aid of soil auger. The samples were collected in well-labeled polythene bags and then transported to the Microbiology Laboratory at The Federal University of Technology, Akure.

Physicochemical analysis of soil sample

The physicochemical properties of the soils such as pH, soil textures, organic carbon, nitrogen, potassium, phosphorus, and calcium were assessed. A total of fourteen (14) soil properties were assessed, including pH, organic carbon (C), organic nitrogen (N), cation exchange capacity (CEC), water holding capacity (WHC), soil texture, and bulk density (BD). Soil pH was measured by adding 10 ml of distilled water to 4g of soil and recording pH using a pH electrode. Analysis of organic carbon and was performed using an Elemental Analyzer and a High Performance Microflow Analyze (Tao et al. 2016).

Isolation of fungal isolates

A six-fold serial dilution was carried out on rhizosphere soil samples of cocoa and orange trees with sterile distilled water. An aliquot (0.5 ml) from 4^{th} and 6^{th} placed on sterile potato dextrose agar (PDA) containing 1% chloramphenicol using pour plate method. The experiment was done in triplicates, and plates were incubated at 28˚C for 72 hours (Seth et al. 2016). The Fungal count was estimated for each sample of the soil, and this was done by counting the total number of colony counts present in the plates. Distinct colony count on the culture plates were sub-cultured until pure cultures of different isolates were obtained. The pure isolates were transferred into PDA slant in McCartney bottles and kept in the refrigerator at 4˚C as stock culture.

Identification of fungal Isolates

The colony of fungal isolates was observed macroscopically based on morphological features such as colour, shapes and sizes. The staining technique was used for microscopic identification. This was done by

putting a drop of lactophenol blue on a slide using a sterile inoculating needle. A small portion of mycelium of each fungal isolate was then picked with the needle and teased with lactophenol cotton blue. The stain was covered with slip and examined under × 40 objective lens of the compound microscope (Sohail and Bayan, 2018).

Production of fungal biomass

The fungal isolates were cultured on potato dextrose broth at 28°C on a shaker incubator (Japan M56) (at 120 rpm) for 72 hours. The biomass was collected after period of incubation and was centrifuged at 2,500 rpm for 5 minutes. The supernatant was collected and used for the biosynthesis of silver nanoparticles according to previously published method (Mohamed et al. 2014).

Biosynthesis and characterization of silver nanoparticles

1 g of silver nitrate ($AgNO_3$) was introduced into 500 ml of distilled water and it was filtered using a membrane filter. Forty milliliters (40 ml) of $AgNO_3$ solution (0.1 mM) was mixed with 40 ml of supernatant (cell filtrate) and a reaction mixture without $AgNO_3$ was used as control. The prepared solutions were incubated at 28°C for 94 hours. All solutions were kept in the dark to avoid any photochemical reactions during the experiment as described earlier (Mohamed et al. 2014).

Determination of absorbance spectrum of synthesized silver nanoparticles using UV-visible spectroscopy

UV-V is spectroscopy is the most important technique and the simplest way to confirm the formation of nanoparticles. The absorbance spectrum of the colloidal sample was obtained in the range of 252-652 nm, using a UV-Vis spectrometer Shimadzu-UV 1800 (Perkin-Elmer Lamda 35, Germany) with distilled water as a reference according to Anandalakshmi et al. (2016). After the addition of $AgNO_3$ to the supernatant of the biomass, the absorbance was taken after the formation of colour change from light yellow to dark brown by periodic sampling of aliquots (0.1 ml) of aqueous component and measuring UV-Vis spectra of the solution (Anandalakshmi et al. 2016).

Determination of functional groups in synthesized silver nanoparticles using Fourier Transformation Infrared (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds (Mohamed et al. 2014). The wavelength of light absorbed is characteristic of the chemical bond. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. A FTIR spectrometer (Infrared spectrometer Varian 660 MidIR Dual MCT/DTGS Bundle with ATR) was used to confirm the chemical structure of all samples. Before analysis, the samples were dried in an auto- desiccator for 24 hours. Samples were directly applied to a diamante crystal of ATR and resulting spectra of them were used for background air absorbance. Potassium bromide (KBr) disks were prepared from powdered samples mixed with dry KBr in the ratio of 1:100. The spectra were recorded in a transmittance mode from 4000 to 500/400 cm^{-1} at a resolution of 4 cm according to (Anandalakshmi et al. 2016).

Statistical analysis

Data obtained from this study were subjected to descriptive statistics. One-way analysis of variance (ANOVA) was carried out and means were separated by Duncan's New Multiple Range test using SPSS version 22, at $p \leq 0.05$ level of significance.

Results

Physicochemical properties of soil samples

The pH of orange and cocoa rhizosphere was slightly acidic but that of orange (5.41) was higher than that of cocoa (4.99). In addition, the percentage of organic matter, organic carbon and clay of orange were also higher than that of cocoa. Orange rhizosphere was sandy clay loam while that of cocoa was loam (Table 1).

Table 1: Physicochemical characteristics of the soil samples.

Parameter	Cocoa Rhizosphere	Orange Rhizosphere
pH	4.99±0.07^{a}	5.41±0.01^{b}
OM (%)	4.48±0.01^{a}	7.30±0.01^{b}
OC (%)	2.60±0.01^{a}	4.23±0.01^{b}
WHC (%)	49.20±0.71^{b}	46.32±0.54^{a}
Sand (%)	39.73±0.66^{a}	44.00±1.83^{a}
Silt (%)	35.64±0.11^{b}	25.28±0.26^{a}
Clay (%)	24.75±0.47^{a}	30.76±1.53^{b}
Soil texture	Sandy loam	Sandy clay loam
N (mg/kg)	0.43±0.02^{a}	0.41±0.01^{a}
P (mg/kg)	0.46±0.01^{a}	0.43±0.03^{a}
K (mg/kg)	3.70±0.02^{a}	3.65±0.12^{a}
Ca (mg/kg)	3.62±0.08^{a}	4.14±0.14^{a}
CEC (mol/kg)	18.00±0.43^{b}	16.77±0.16^{b}
BD (g/cm^{2})	1.06±0.07^{a}	1.04±0.01^{a}

Values are presented as Mean ± S.E (n =3). Values with the same superscript letter(s) along the same row are not significantly different ($p<0.05$). pH = Potential of Hydrogen; OM = Organic Matter; WHC = Water Holding Capacity; N = Nitrogen; P = Phosphorus; K = Potassium; Ca = Calcium; CEC = Cation-exchange capacity.

Identification of fungi isolated from rhizosphere of cocoa and orange

Seven fungi isolated from rhizosphere of cocoa were *Nigrospora sphaerica, Penicillium discolor, P. digitatum, Alternaria infectoria, Trichophyton verrucosum* and *Mucor mucedo.* Two of the above isolates absent in the rhizosphere of orange were *N. sphaerica* and *T. verrucosum*.

Spectra of the biosynthesized silver nanoparticles by fungi

The absorbance of silver nanoparticles synthesized by *N sphaerica* at wavelength of 440nm was 1.645 (Fig. 1) while that of *N. crassa* was 0.383 at the same wavelength (Fig. 2). At wavelength of 420nm, AgNPs synthesized by *A infectoria* had absorbance of 1.602 (Fig. 3); *P. digitatum* 1.533 (Fig. 4), *T. verrucosum*

0.994 (Fig. 5), *M. mucedo* 0.790 (Fig. 4.6) and *P. discolor* 0.415 (Fig. 7). However, the NPs synthesized by *A. infectoria* had the highest absorbance than those synthesized by other fungi at 420 nm.

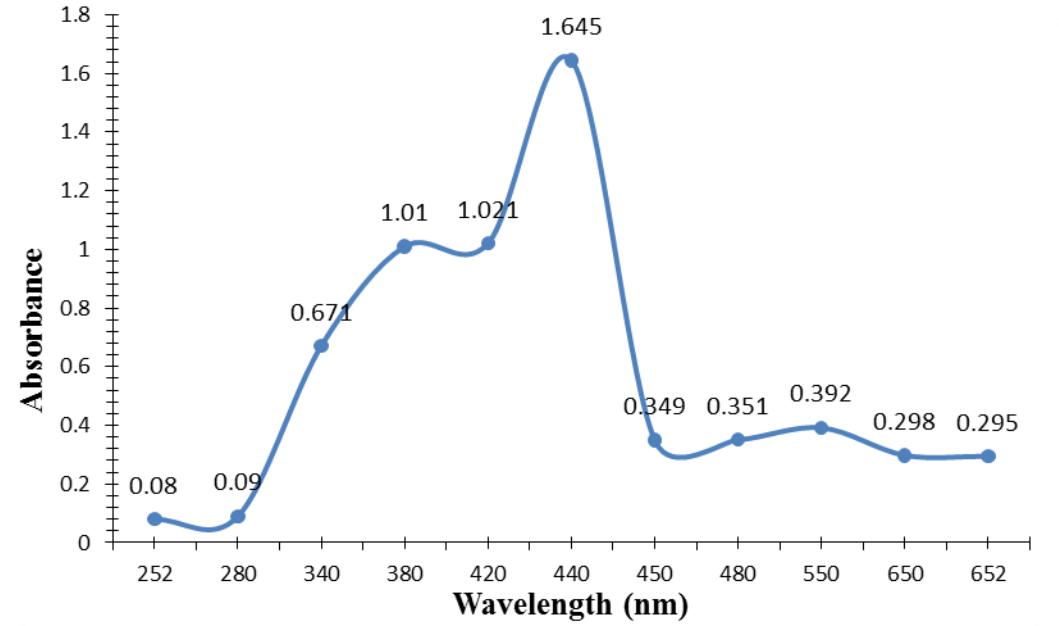

Fig. 1: UV–VIS spectra of the synthesized silver nanoparticles by *N. sphaerica.*

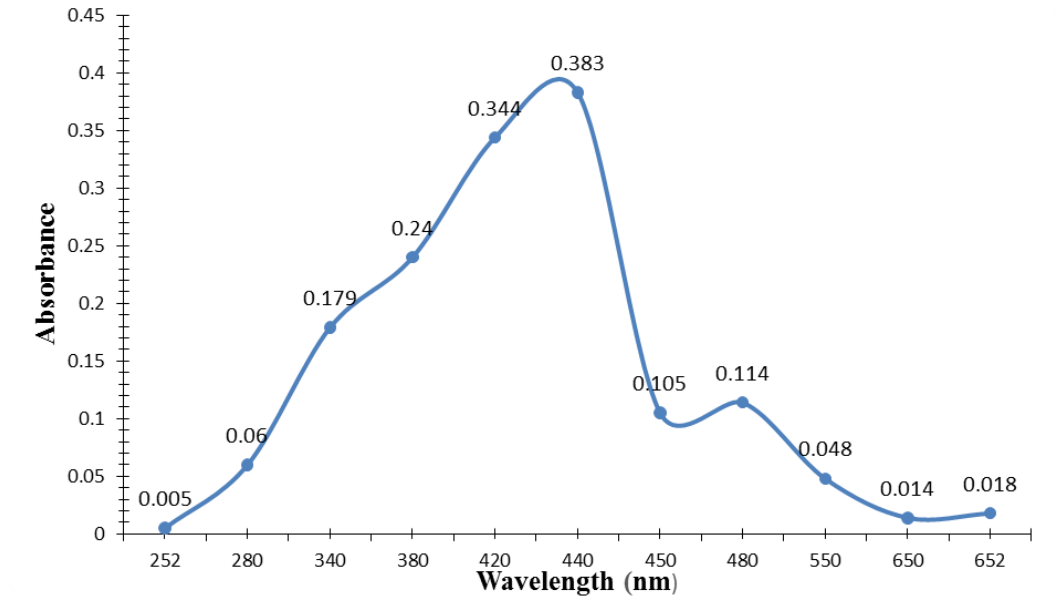

Fig. 2: UV–VIS spectra of the synthesized silver nanoparticles by *N. crassa.*

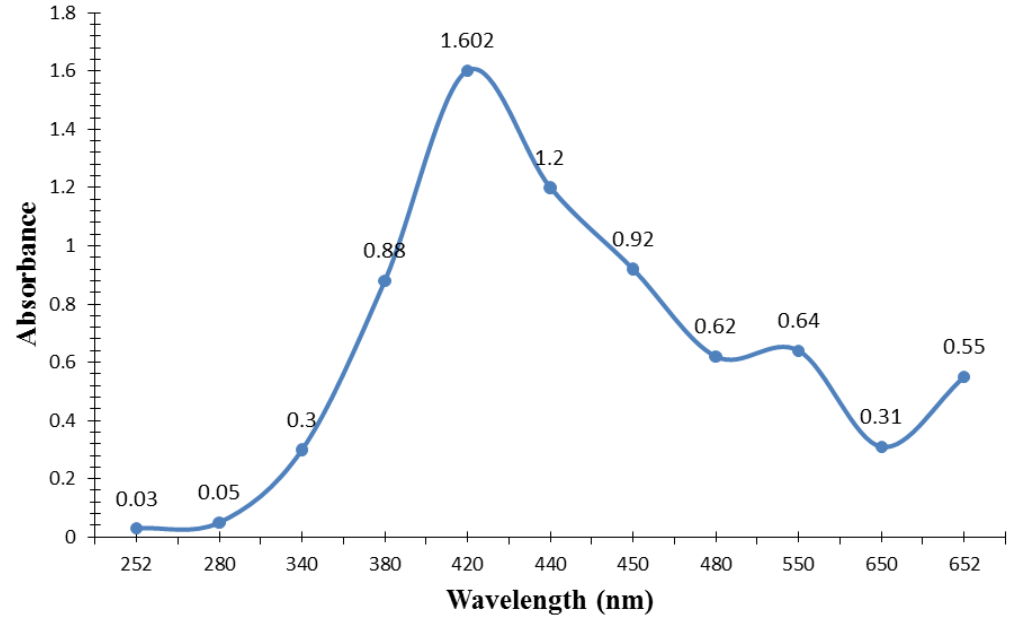

Fig. 3: UV–Vis spectra of the synthesized silver nanoparticles by *A. infectoria.*

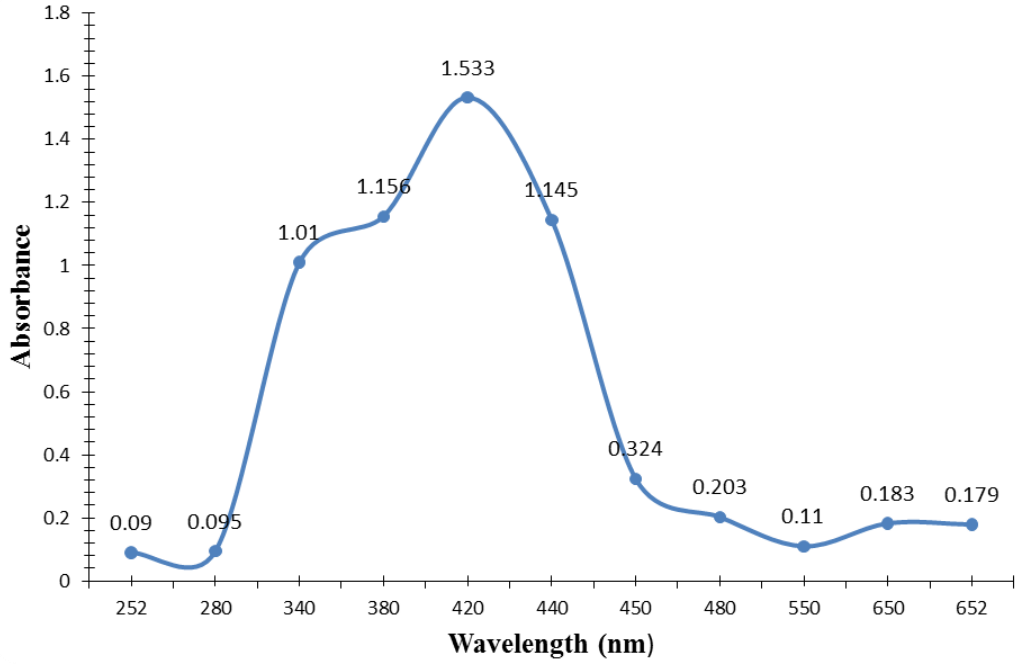

Fig. 4: UV–VIS spectra of the synthesized silver nanoparticles by *P. digitatum.*

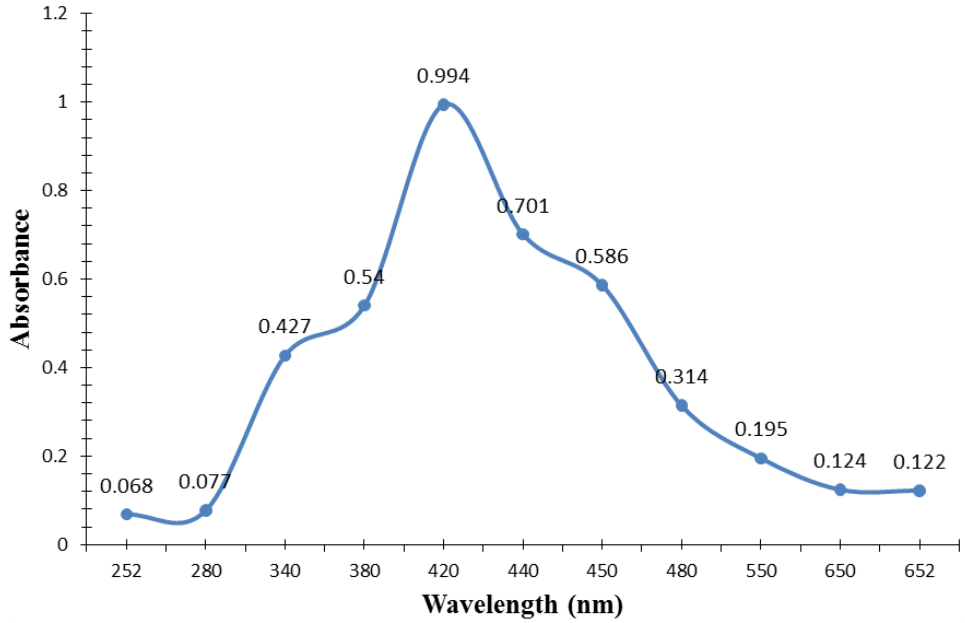

Fig. 5: UV-VIS spectra of the synthesized silver nanoparticles by *T. verrucosum.*

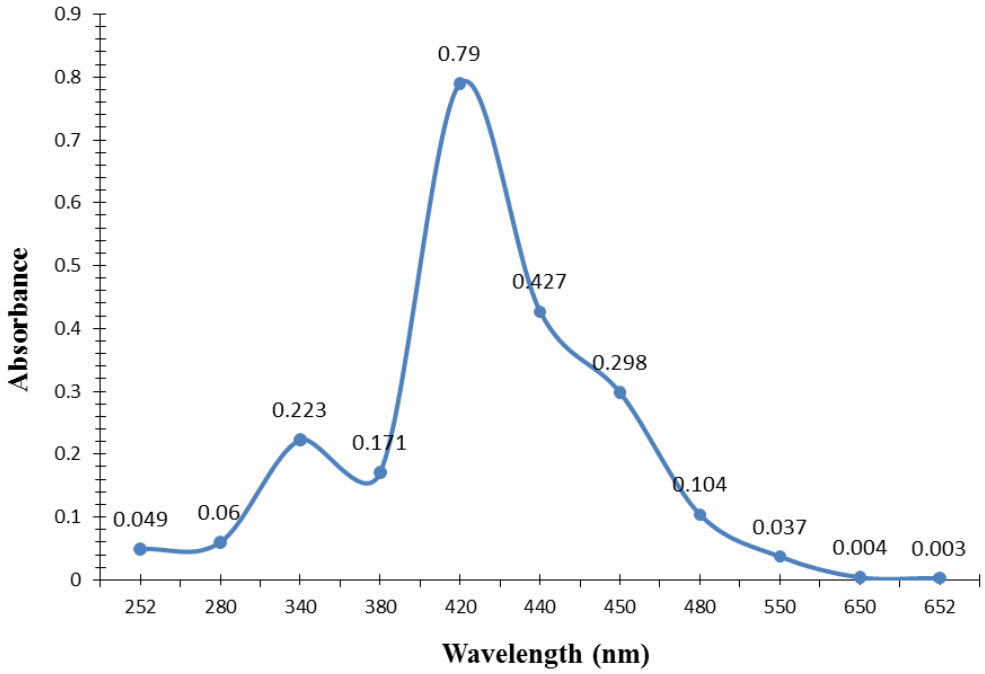

Fig. 6: UV-VIS Spectra of the synthesized silver nanoparticles by *M. mucedo.*

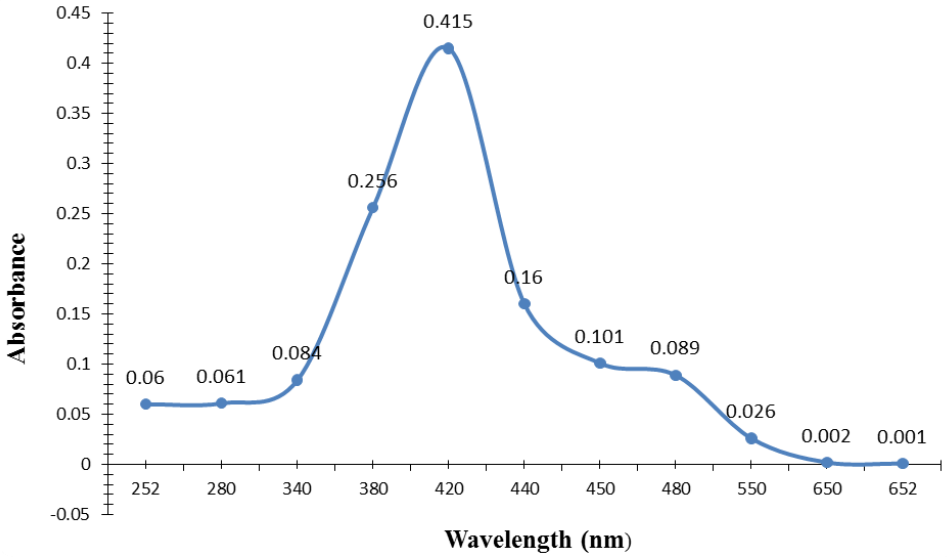

Fig. 7: UV-VIS spectra of the synthesized silver nanoparticles by *P. discolor.*

FT-IR spectra of the biosynthesized silver nanoparticles

At an absorbance of 70, wavelength of *N. sphaerica* was 3514.36 cm-1 (Fig. 8) while that of *P. discolor* was 3310.47 cm-1 (Fig. 9). at absorbance 98, the wavelength of *P. digitatum* was 2412.22 cm-1 (Fig. 10), *N. crassa* was 3912.30 cm-1 (Fig. 11), *A. infectoria* was 2000 (Fig. 12) and *T. verrucosum* was 3852.11 (Fig. 13). Also, at absorbance at 45, the wavelength of *M. mucedo* was 2112.22 (Fig. 14).

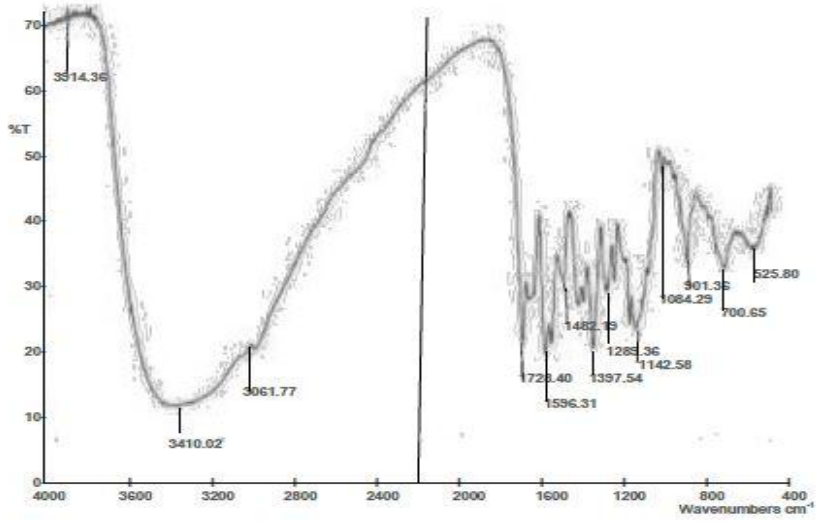

Fig. 8: FT-IR Spectra of silver nanoparticles synthesized by *N. sphaerica.*

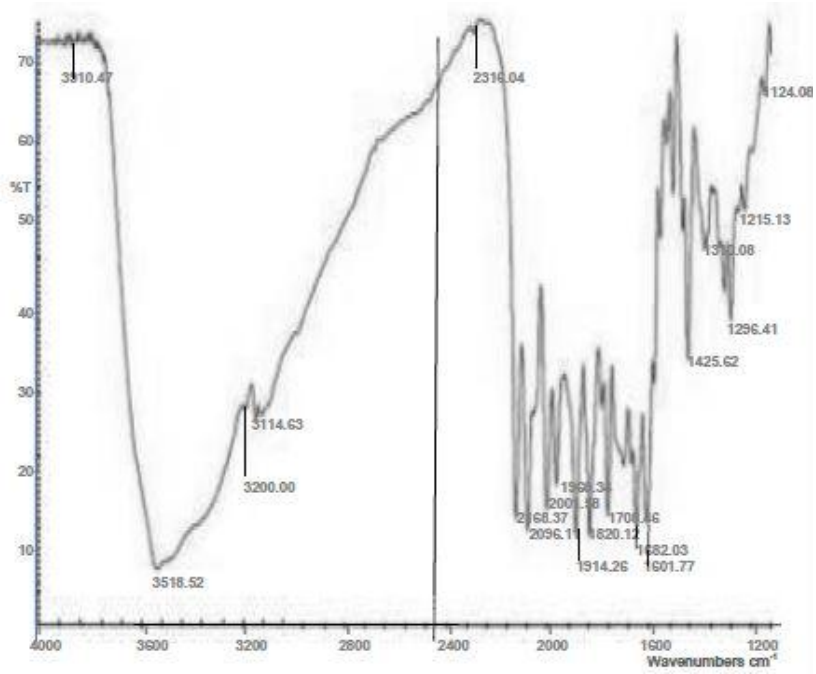

Fig. 9: FT-IR spectra of silver nanoparticles synthesized by *P. discolor.*

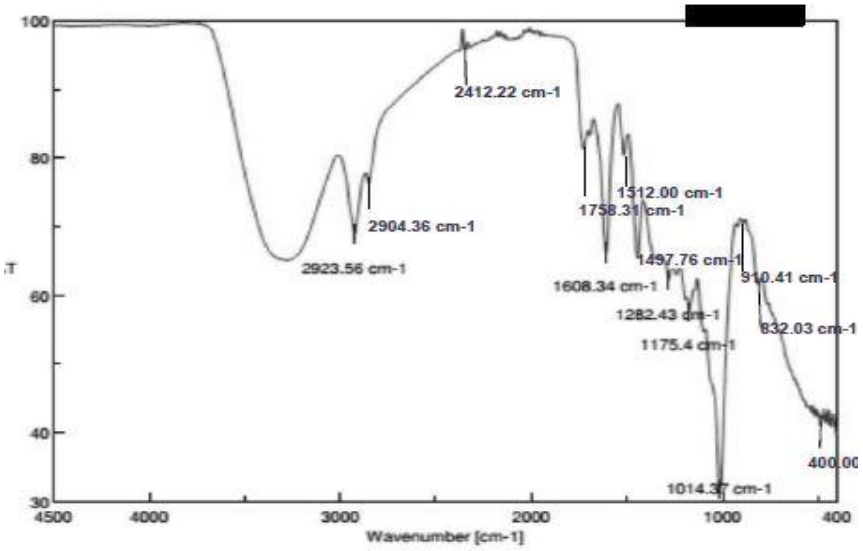

Fig. 10: FT-IR Spectra of silver nanoparticles synthesized by *P. digitatum*.

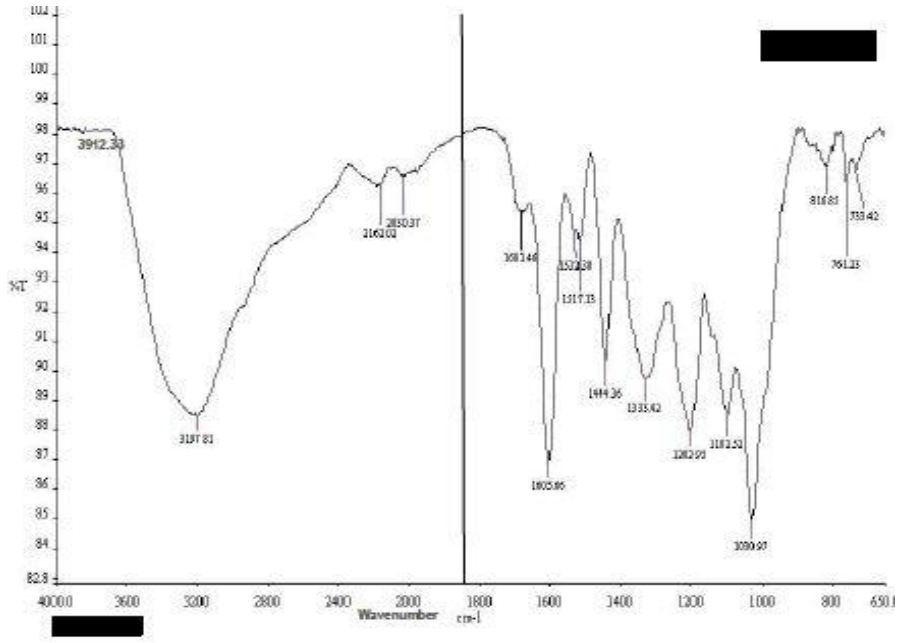

Fig. 11: FT-IR Spectra of silver nanoparticles synthesized by *N. crassa.*

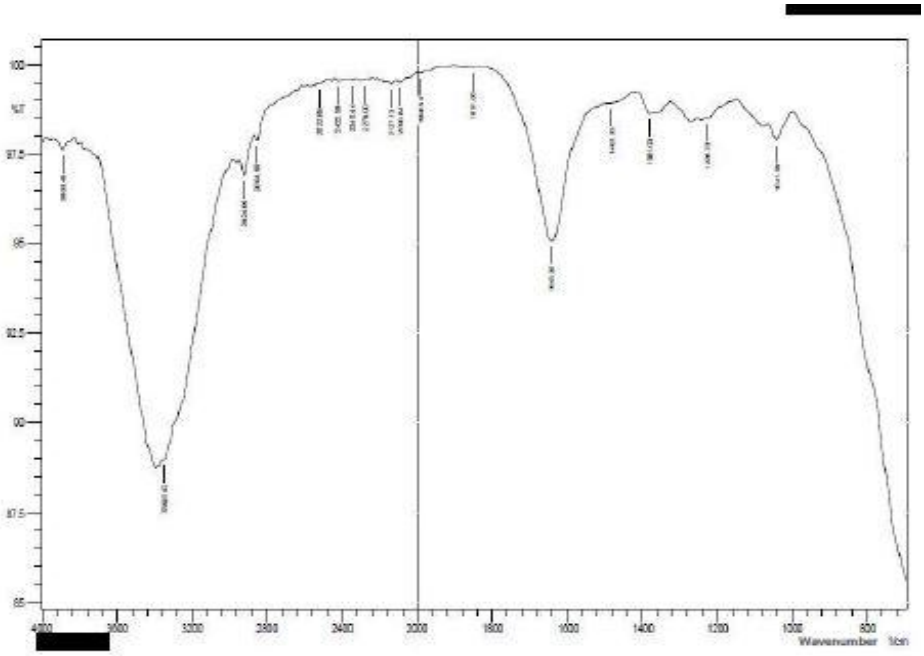

Fig. 12: FT-IR spectra of silver nanoparticles synthesized by *A. infectoria.*

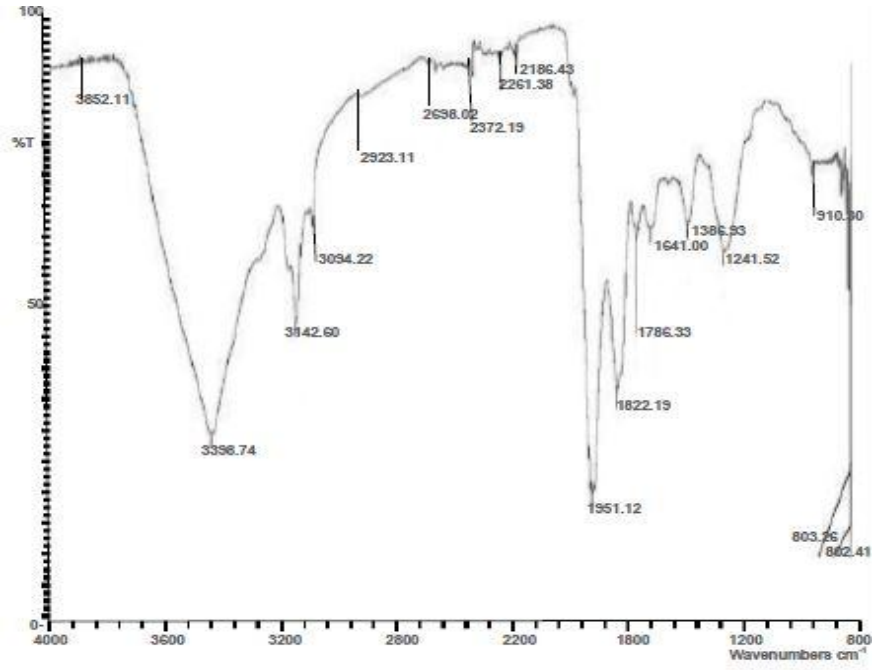

Fig. 13: FT-IR spectra of silver nanoparticles synthesized by *T. verrucosum.*

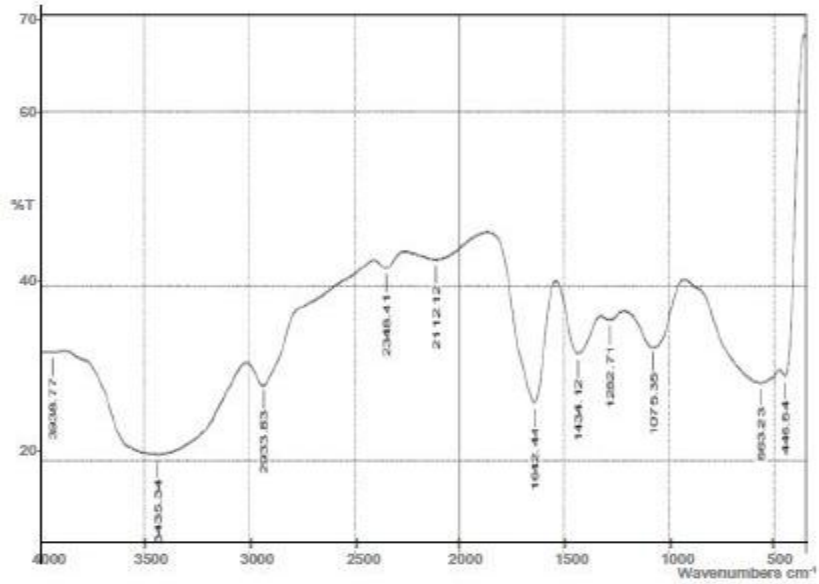

Fig. 14: FT-IR spectra of silver nanoparticles synthesized by *M. mucedo.*

Discussion

Soil moisture is assumed to be very important for microorganisms, because water availability is fundamental for different processes. The soil pH has a strong influence on species richness of soil fungi, diversity, and community structure (Ferrol et al. 2004, Ihuma et al. 2022). The composition and proportion of the soil components have appreciable effects on nutrient concentrations and soil texture, thereby influencing the community of soil fungi (Lauber et al. 2008, Ihuma et al. 2022). Soil fungi are an immensely diverse group of organisms, and their diversity is affected by the local environmental conditions (Tardy et al. 2015), including the chemical and physical soil characteristics, which determine to a great extent the composition of fungal communities as reported by Requena et al. (2001) and Ihuma et al. (2022). Soil degradation is triggered by human activities (anthropogenic), which influence the biodiversity of soil as stated by Eswaran et al. (2001); Ihuma et al. (2022), and it impacts fungal diversity because soil characteristics influence the presence, distribution, and abundance of fungal species, and the soil characteristics depend on the soil degradation level. Every soil particle has a different micro-spatial composition of fungal species, which is influenced by different micro-habitats in the soil (Gibbs and Salmon 2015). The fungal isolates obtained from the rhizosphere of cocoa and plantation soil were identified as *N. sphaerica, P. discolor*, *P. digitatum, N. crassa, A. infectoria, T. verrucosum* and *M. mucedo.* These variations in the occurrence of fungi isolated have been reported. Zako et al. (2012) stated that the variation could be due to environmental factors and soil physico-chemical characteristics which affect the density, distribution and composition of fungal spores. The occurrence of these microorganisms in rhizosphere of all the soil samples examined is in conformity with what has been reported by Wolfe and Kilironomas (2005). Similarly, it was reported that the quality of root exudates promotes the differential recruitment of microorganisms present in the soil. Also, the number and quality of fungi isolated in rhizosphere of cocoa and plantation soil in this study is similar to fungi such as *P. digitatum, A. infectoria,* and *M. mucedo* reported by Ravindra et al. (2013). Ihuma et al. (2022) recently isolated fungal species including *Aspergillus*, *Mucor*, *Cladosporium*, *Fusarium* and *Aspergillus*.

Characterization of the myco-synthesized silver nanoparticles by UV-Visible spectrometer shows that a strong and narrow surface plasmon absorption peak (SPR) was observed at wavelengths 400-440 nm, which agrees with the research of Adewumi et al. (2018) which stated that silver nanoparticle is formed within the wavelength range of 400-490 nm with the formation of the ideal bell shape which is characteristic for the formation of Ag^0 nanoparticles. The surface plasmon resonance comes from the free electron arising from the conduction and valence bands lying close to each other in metal nanoparticles. It is as a result of the collective oscillation of free electron of silver nanoparticles in resonance with the light wave in silver nanoparticle synthesis (Marwa et al. 2018).

Mohamed et al. (2014) stated that fungal cell filtrate treated with silver nitrate solution is known to show a peak around 440 nm with high absorbance which supports the finding of the peaks observed for absorbance at 440 nm, indicating the biosynthesis of nanoparticles by *A. ochraceus*, which is also synonymous with silver nanoparticles biosynthesized in this study by *A. infectoria*., *P. digitatum*, *N. sphaerica* and *T. verrucosum* which show peak near 440 nm with high absorbance while *M. mucedo., N. crassa* and *P. discolor* show low absorbance near 420 nm.

Conclusion

The findings of this study have shown that seven fungal isolates belonging to six (6) different genera vis; *N. sphaerica, Penicillium* species*, N. crassa, A. infectoria, T. verrucosum* and *M. mucedo* were recovered from the rhizosphere of cocoa and orange trees and these isolates were able to synthesize silver nanoparticles (AgNPs). The silver nanoparticles synthesized could have the potential application in agricultural,

pharmaceutical and food industries as well as petrochemical industries for the clean-up of crude oil contaminated soils.

Competing interest: Authors have none to declare.

Funding: None received.

References

Achmad S, Salmiati, Mohd, RS, Ahmad BH, Tony H and Hadi N (2017). A review of silver nanoparticles: research trends, global consumption, synthesis, properties, and future challenges. Journal of the Chinese Chemical Society 64: 732-756.

Adewumi OD, Folahan AA, Oluyomi SA, Oluwasesan MB, Adetunji CO, Oluwakemi JA and Femi-Adepoju AG (2018). Exploring the effect of operational factors and characterization imperative to the synthesis of silver nanoparticles. International Journal of Separation for Environmental Sciences 1(1): 253-261.

Anandalakshmi K, Venugobal J and Ramasamy V (2016). Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity. Applied Nanosciences 6: 399-408.

Awwad A, Salem N and Abdeen A (2013). Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. International Journal Industrial Chemistry 4(1): 1-6.

Eswaran H, Lal R and Reich PF (2001). Land degradation. An overview conference on land degradation and desertification. Proceedings 2nd International Conference on Land Degradation and Desertification; Khon Kaen, Thailand; New Dehli, India: Oxford Press.

Ferrol N, Calvente R and Cano C (2004). Analysing arbuscular mycorrhizal fungal diversity in shrub-associated resource islands from a desertification threatened semiarid Mediterranean ecosystem. Applied Soil Ecology 25(2): 123-133.

Fouda A, Saad E, Salem SS and Shaheen TI (2018). *In vitro* cytotoxicity, antibacterial, and UV protection properties of the biosynthesized zinc oxidenanoparticles for medical textile applications. Microbial Pathogenesis 125: 252-261. doi: 10.1016/j.micpath.2018.09.030

Gibbs H and Salmon J (2015). Mapping the world's degraded lands. Applied Geography 57: 12-21.

Goutam SP, Saxena G, Roy D, Yadav AK and Bharagava RN (2020). Green synthesis of nanoparticles and their applications in water and wastewater treatment. *In*: Bioremediation of Industrial Waste for Environmental Safety, eds Saxena G and Bharagava RN (Basingstoke: Springer Nature), pp. 349-379.

Ihuma JO, Agida IO and Nashima TN (2022). Assessment of Fungi Species Associated with a Multicultural Orchard and Cultivated Land in Bingham University Landscape, Karu Nasarawa State, Nigeria. Research in Ecology 4(1): 7-13

Keat CL, Azila A, Ahmad ME and Nagib AE (2015). Biosynthesis of nanoparticles and silver nanoparticles. Bioresource and Bioprocessing 2: 47-61.

Lauber C, Strickland M and Bradford M (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry 40(9): 2407-2415.

Marwa E, Sahar Z, Marwa, E and Desouky A (2018). Biosynthesis, characterization of some combined nanoparticles, and its biocide potency against a broad spectrum of pathogens. Hindawi Journal of Nanomaterials article (1): 1-16.

Mohamed HE, Hany MM and Marwa MA (2014). Biosynthesis of silver nanoparticles using fungi and biological evaluation of mycosynthesized silver nanoparticles. Egypt Journal Experiment Biology 10(1): 1-12.

Mohanpuri P, Rana NK and Yadav SK (2008). Biosynthesis of nanoparticles, technological concepts and future applications. Journal of Nanoparticle Research 10: 507-517.

Ravindra K, Asha S, Seweta S and Manisha S (2013). Effect of green manuring of *Sesbania aculeata* L. on rhizosphere microflora of okra (*Abelmoschus esculentus* L.). Crop Resource Paper 46(1, 2 and 3): 200-204.

Requena N, Pérez-Solís E and Azcón-Aguilar C (2001). Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Applied and Environmental Microbiology 67(2): 495-498.

Saba H (2015). A review on nanoparticles: their synthesis and types. Research Journal of Recent Sciences (4): 1-3.

Seth RK, Alam S and Shukla DN (2016). Isolation and identification of soil fungi from wheat cultivated area of Uttar Pradesh. Journal of Plant Pathology and Microbiology 7(11): 384.

Singh P, Kim YJ, Zhang D and Yang DC (2016). Biological synthesis of nanoparticles from plants and microorganisms. Trends in Biotechnology 34: 588-599. doi: 10.1016/j.tibtech.2016.02.006

Slavin YN, Asnis J, Hafeli UO and Bach H (2017). Metal nanoparticles: understanding the mechanisms behind antibacterial activity. Journal of Nanobiotechnology 15: 65.

Sohail AA and Bayan MB (2018). Morphological and molecular identification of fungi isolated from different environmental sources in the northern eastern desert of Jordan. Jordan Journal of Biological Sciences (11): 329-337.

Spagnoletti FN, Spedalieri C, Kronberg F and Giacometti R (2019). Extracellular biosynthesis of bactericidal Ag/AgCl nanoparticles for crop protection using the fungus *Macrophomina phaseolina*. Journal of Environmental Management 231: 457-466. doi: 10.1016/j.jenvman.2018.10.081

Tao Z, Neng-Fei W, Hong-Yu L, Yu-Qin Z and Li-Yan Y (2016). Soil pH is a key determinant of soil fungal community composition in the Ny-Ålesund region, Svalbard (high arctic). Frontier in Microbiology 7: 227-229.

Tardy V, Chabbi A and Charrier X (2015). Land use history shifts in situ fungal and bacterial successions following wheat straw input into the soil. PLoS One 10(6): e0130672-17.

Valgaz APF, Naranjo-Moran J, Roman GR, Oviedo-Anchundia J, Torres MR and Barcas-Arias M (2022). Discovering the diversity of arbuscular mycorrhizal fungi associated with two cultivation practices of *Theobroma cacao*. *Diversity. 14*: 651. https://doi.org/10.3390/d14080651

Wolfe BE and Klironomos JN (2005). Breaking new ground: Soil communities and exotic plant invasion. BioScience 55(6): 477-487.

Xiangqian L, Huizhong X, Zhe-Sheng C and Guofang C (2011). Biosynthesis of nanoparticles by microorganisms and their applications. Journal of Nanomaterials 1: 1-16.

Zako MS, Tié BT, Zirihi GN, Kouadjo ZG, Fossou KR and Adolphe Z (2012). Arbuscular mycorrhizal fungi associated with *Theobroma cacao* L. in the region of Yamoussoukro (Cote d'Ivoire). African Journal of Agricultural Research 7(6): 993-1001.

(Manuscript received on 13th April 2024 and revised on 17th May 2024)