Research Article **CODEN: IJRPJK** **ISSN: 2319 – 9563**

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: **www.ijrpns.com**

GREEN BIOSYNTHESIS OF COPPER OXIDE NANOPARTICLES USING SOME FUNGI ISOLATED FROM THE EGYPTIAN SOIL

Mohamed Ghareib*[1], Medhat Abu Tahon[1], Wafaa E. Abdallah[1], Abubakr Tallima[2]

[1*]Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Roxy 11757, Cairo, Egypt.

[2]Ministry of Education, Egypt.

ABSTRACT

Copper oxide nanoparticles are of significant importance in many fields of application especially in the biomedical field. Green biosynthesis of these nanoparticles using fungi had not received the necessary attention from researchers despite their excellent fermentation characteristics. This work was a trial to bridge the gap in this respect. Out of nineteen fungal species isolated from the Egyptian soil, only six species could synthesize these nanoparticles using their culture supernatant and/or biomass. *Aspergillus fumigtus* was confirmed as good candidate for biosynthesis of copper oxide nanoparticles. It could produce the highest yield of nanoparticles when the preformed mycelium was contacted with 1 mM solution of copper nitrate solution adjusted to pH 6 and incubated in the dark at 30°C for 60 h under submerged conditions. The biosynthesized nanoparticles showed characteristic absorption spectrum with intense peak at 335 nm. The nanoparticles were spherical in shape when examined by high resolution transmitting electron microscope. Copper oxide nanoparticles of *A. fumigatus* were suggested as good free radical scavengers.

KEYWORDS

Copper oxide, Nanoparticles, Fungi, Free radical and Scavenging.

Author for Correspondence:

Mohamed Ghareib,
Department of Biological and Geological Sciences,
Faculty of Education,
Ain Shams University, Cairo, Egypt.

Email: mohaghareib@yahoo.com

INTRODUCTION

Metallic nanoparticles (NPs) are being viewed as the future material and represent the starting point for nanostructured materials and devices[1]. These NPs have gained great interest in the last decades due to their unique physical and chemical properties leading to their potential use in a wide range of applications. In addition, they also exhibit unique electronic, magnetic, catalytic and optical properties that are different from those of bulk metals[2]. The

metal oxide NPs has entered the scientific spot-light in the last years. Among transition metals, interest is being shown to copper (Cu) and copper oxides (CuO and Cu_2O) NPs because of their unique properties and potential applications. They have numerous scientific and technological applications to our day life. Of these applications, use of the green synthesized CuO NPs as alternative to the antioxidants is of prime interest in fighting microbial pathogens. It is well known that many of the pathogenic microorganisms acquired their pathogenicity by generation of free radicals (FRs)[3] leading to damaging of the host cell walls and cell membranes. Role of the antioxidants is proposed to scavenge the FRs to prevent the disease progress[4]. CuO NPs are traditionally produced through physical[5-7] and chemical protocols[8-10] although their low efficiency with regard to environmental and economic parameters. Biological methods have been avoided drawbacks of the chemical and physical methods and suggested as possible eco-friendly alternatives for the synthesis of low-cost, energy efficient and non-toxic CuO NPs. Most published works on the green biosynthesis of CuO NPs used the plant extracts[11-14]. A rare published works were found to utilize different microorganisms including algae[15], bacteria[2] and fungi[16,17]. This lack of published research work on the use of fungi for the biosynthesis of CuO NPs is not justified. Fungi are characterized by their ease of cultivation, requirements of mild experimental conditions, easy of downstream processing in addition to their secretion of large amounts of enzymes. So, this work was planned to study the potentiality of some fungi isolated from the Egyptian soil as producers of CuO NPs. Optimization of the biosynthesis of these NPs by the most potential fungus and utilization of the synthesized CuO NPs for scavenging the 2,2-diphenyl-1-picrylhydrazil (DPPH) has been also investigated.

MATERIAL AND METHODS

Chemicals and glasswares

All chemical used were of analytical grade. All reagent solutions were made with deionized water. CuO NO_3 was purchased from Sigma. The glasswares were washed with aqua regia (mixture formed by freshly mixing concentrated nitric acid and hydrochloric acid in a volume ratio of 1:3) and then thoroughly rinsed with deionized water to remove any metal contaminant.

Organisms

The used fungi were isolated from soil samples collected from certain localities of Egypt, identified by Assiut University Mycological center (AUMC) where they were deposited with their accession numbers. The isolated fungi were grown and maintained on Czapek's- Dox agar medium at 30^{o}C and sub-cultured whenever required.

Cultivation of fungi

Triplicate sets of 250 ml flasks each containing 50 ml of the Czapek's broth having the following constituents (g/100ml): sucrose, 3; $NaNO_3$, 0.2; KH_2PO_4, 0.1; KCl, 0.05; $MgSO_4.7H_2O$, 0.05 and $FeSO_4.5H_2O$, 0.001were used. The flasks were sterilized, left to cool, initially adjusted to pH 6 and inoculated with 1 ml of fungal spore suspension containing about 10^6 spores and the cultures were incubated at 30^{o}C on rotary shaker adjusted at 150 rpm for 72 h. By the end of the incubation period, the biomass was separated from the culture supernatant (CS) by filtration through Whatman filter paper No.1. The CS of any fugal isolate was collected, centrifuged at 3000 rpm for 10 min. On the other hand, the biomass was washed extensively with sterile deionized water to remove all possible medium components. Both CS and biomass of any fungal isolate were separately used as the starting material for synthesis of CuO NPs.

Extracellular biosynthesis of CuO NPs

Using the CS

Triplicate sets of 250 ml Erlenmeyer flasks each containing 90 ml of the CS and10 ml of 10 mM copper nitrate ($Cu(NO_3)_2.3H_2O$) in deionized water was added and mixed well so the final concentration would be 1mM. Simultaneously, a positive control

(CS) and negative control (1mM Cu $(NO_3)_2.3H_2O$) were also checked for comparison. All sets were kept under agitation (150 rpm) at 30ºC in the dark.

Using the washed biomass

Typically 10 g of biomass (fresh weight) were directly brought in contact with 90 ml of 1mM Cu $(NO_3)_2.3H_2O$, kept on the rotary shaker and completed as described before. Both positive (biomass in deionized water) and negative controls were run along with the experimental flasks.

Optimization of the reaction conditions

Influence of pH values of the reaction mixture (fungal biomass in 1mM $Cu(NO_3)_2.3H_2O$) on the biosynthesis process was studied by adjusting pH of different sets of flasks containing the reaction mixture as well as the two controls in the range of 5-10 and the work was completed as above. To study the effect of reaction temperature on formation of the CuO NPs, the reaction mixtures adjusted at pH 6 along with the controls were separately incubated at different temperatures under the previously specified conditions. The effect of changing the salt concentration was studied in the specified range. In the last step, biosynthesis of the investigated NPs was followed at different periods of incubation under the best favorable conditions.

Characterization of CuO NPs

Visual observations

The reduction of copper ions (Cu^{2+}) was routinely monitored by visual inspection. Change in the colloidal solutions towards green (or greenish) color was taken as preliminary sign of CuO NPs formation.

UV-Vis spectroscopy

To confirm the formation of CuO NPs, the UV-Vis spectrum of the reaction medium showing a change in color was monitored after filtration through 0.22 µm membrane filter (Millex-GS, Millipore, Madrid, Spain). Absorption measurements were carried out at wavelengths from 200 to 800 nm using a double beam spectrophotometer (Metash UV-Vis, model UV-8500) at a resolution of 1 nm.

High Resolution-Transmission Electron Microscopy (HR-TEM)

Morphology of the CuO NPs was performed in central lab of national research center (NRC), Dokki, Giza, Egypt. For this purpose, an aliquot of an aqueous suspension of CuO NPs was transferred onto a carbon coated copper grid. Samples were dried and kept under vacuum in desiccators before loading them onto a specimen holder. The grid was then scanned using a Jeol JEM-2100 (Made in Japan Model Year 2000) operated at a voltage of 200 kV.

2,2-Diphenyl-1-picrylhydrazil (DPPH) radical scavenging assay

The free radical scavenging activity (RSA) of the CuO NPs was examined *in vitro* using DPPH radical as described by Shimada *et al.*[19]. When the stable DPPH radical accepts an electron from the antioxidant material, its color is reduced from violet to yellow diphenylpicrylhydrazine radical that measured colorimetrically. Ascorbic acid was used as standard antioxidant agent. The DPPH radical scavenging activity (RSA) was expressed in percentage of inhibition using the following formula:

$$RSA = [AC- AS] / AC \times 100$$

Where: AC is the absorbance of the blank control and AS is that absorbance of the test sample.

RESULTS AND DISCUSSION

Screening

In this work, screening program was used to examine potentiality of nineteen fungal isolates for the extracellular biosynthesis of CuO NPs. The isolated fungi were grown on Czapek's medium in submerged cultures for 72 h and the biomass was separated from the CS for each fungus. Both of the biomass and CS were separately added to the copper nitrate $[Cu(NO_3)_2.3H_2O]$ and incubated again on rotary shaker at 150 rpm for additional 60 h at 30ºC in the dark The change of color from light blue to light green or greenish was taken as a preliminary sign of CuO NPs biosynthesis.

The results (Table No.1) show clearly that few numbers of fungi could synthesize CuO NPs with

appropriate yield. Three fungi could produce these NPs using their CS solution. They are *Aspergillus aureoterreus, Emericella nidulans* and *Penicillium pinophilum.* Other two fungi *i.e. A. flavus var. columnaris* and *A. sydowii* produced the NPs by their biomass. Moreover, two fungal species (*A. carneus* and *A. fumigatus*) could produce the CuO NPs using both CS and biomass. The color intensity as well as the UV-Vis spectrum of the produced CuO NPs had been investigated. The highest yield of the CuO NPs was detected on using the biomass of the fungus *A. fumigatus*. The absorption spectrum of the produced NPs varied from 315 nm for those produced by *A. carneus* biomass to 360 nm for those synthesized by *P. pinophilum* CS. The peak 335 nm was recorded as the maximum absorption in four different cases *i.e.* the biomass of *A. flavus var. columnaris,* and *A. sydowii* in addition to the NPs produced by CS and biomass of *A. fumigatus.* Type of peaks also varied from intense as in case of *A. fumigatus* biomass to sharp in case of *P. pinophilum* CS and broad in cases of *A. carneus* biomass**,** *A. sydowii* biomass and *E. nidulans* CS.

For the first time, the fungus *A. fumigatus* Fresenius AUMC 13024 was found relatively suitable in this work for the biosynthesis of CuO NPs. It was isolated from soil sample collected near Giza city in Giza Governorate. The color change from colorless to light green was observed when the preformed biomass of *A. fumigatus* contacted with copper nitrate as precursor indicating the formation of CuO NPs. UV-Vis absorption spectrum of the produced NPs showed the formation of an intense peak with maximum absorption at 335 nm corresponding to the surface plasmon resonance (SPR) of CuO NPs. Due to the deficiency of results on CuO NPs from fungi, we obliged to compare the results with those used other sources of synthesis. A broad absorption peak was observed around 365 nm for CuO NPs biosynthesized using extract of *E.coli*[2]. Moreover, other broad absorption peak with maximum at 310 nm was obtained for CuO NPs produced using leaf extract from *Eichhornia*[12]. On the other hand, the CuO NPs synthesized using leaf extract from certain medicinally important plants[20] were found to have SPR absorption band at 220-235 nm in the UV-Vis spectra.

Optimization

Factors affecting the biosynthesis of CuO NPs using the preformed biomass of *A. fumigatus* was then investigated aiming at optimization of the biosynthetic process. pH value of the reaction mixture is a critical factor influencing the NPs formation (Figure No.1). Biosynthesis of CuO NPs by *A. fumigatus* biomass was achieved as optimum near neutrality as their maximal formation was recorded at pH 6. A decrease in the yield was recorded on both sides of the optimum until no NPs formation was detected at pH 10. This pH may be the best favorable for formation of the important biomolecules especially proteins responsible for the bio-reduction of the copper salt to CuO NPs. In this case, both acidic and alkaline pH values may denature such biomolecules. The change of the pH did not affect the maximum absorption of the produced NPs. Effect of reaction temperature in the range of 28-40ºC on the biosynthesis of CuO NPs was demonstrated in Figure No.2. It is evident that the highest synthesis was revealed at 30ºC. A decline in the NPs yield was observed by decreasing or increasing the temperature and at 40ºC no yield was obtained. The biosynthesis process was then followed on using different concentrations of the precursor Cu $(NO_3)_2.3H_2O$ and the results are presented in Figure No.3. Increasing the concentrations from 0.5 mM to 1 mM enhanced the yield of the CuO NPs. Any further increase depressed the biosynthetic process and at 5 mM no NPs were produced. It is of interest to note that at 2 mM a blue shift of the absorption spectrum was observed and changed to 225 nm instead of 335 nm. Also, the peak at this concentration lost many of its intensity. After stabilization of the studied conditions, the biosynthesis was followed at different periods of incubation (Figure No.4). Production of the CuO NPs was initiated sluggishly, but starting to be visible after 24 h with good absorption at 335 nm. The biosynthetic process was accelerates after that as indicated by increase in the

yield and formation of an intense peak at the same wavelength. The maximal yield was recorded after 60 h of incubation. Worthy of mentioning is that no other peaks were recorded in the absorption spectrum indicating purity of the biosynthesized NPs. HR-TEM was used to determine the morphology details of the biosynthesized CuO NPs. The micrograph (Figure No.5) show spherical uniformly distributed NPs with no signs of agglomeration.

DPPH radical scavenging activity

The RSA of the biosynthesized CuO NPs using *A. fumigatus* preformed biomass was examined *in vitro* using DPPH scavenging assay which is widely used to study the radical scavenging property of materials. The maximum scavenging activity of CuO NPs against DPPH was detected when used at 100µg/ml to be 73.65% in comparison with 88.13% for the standard antioxidant ascorbic acid (Figure No.6). The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. It was calculated to be 55 and 31.5µg/ml for the NPs and the standard, respectively. Antioxidant activity of the NPs was rendered to the preferential adsorption of the antioxidant material onto their surface[21]. CuO NPs biosynthesized in this work showed good antioxidant activity with less toxicity thereby can be used as potential candidate for various biomedical applications.

Table No.1: Potentiality of the Isolated Soil Fungi as Producers of CuO NPs

Fungus	Source	Formation of NPs	Yield (Au)	SPR band (nm)	Type of peak
Alternaria alternata (Fries) Keissler AUMC 13015	Biomass	-	-	-	-
	Cs	-	-	-	-
Aspergillus aureoterreus Samson *et al.* AUMC 13006	Biomass	-	-	-	-
	CS	+	0.60	350	Good
Aspergillus carneus Blochwitz AUMC 13007	Biomass	+	0.33	315	Broad
	CS	+	0.50	320	Broad
Aspergillusflavus Link AUMC 8653	Biomass	-	-	-	-
	CS	-	-	-	-
Aspergillus flavus var. columnaris Raper and Fennell AUMC 13012	Biomass	±	0.10	335	Broad
	CS	-	-	-	-
Aspergillus fumigatus Fresenius AUMC 13024	Biomass	++	0.90	335	Intense
	CS	+	0.61	335	Good
Aspergillus niger Van Tieghem AUMC 13022	Biomass	-	-	-	-
	CS	-	-	-	-
Aspergillus sydowii (Bainier and Sartory) Thom and Church	Biomass	+	0.37	335	Broad
	CS	-	-	-	-
Aspergillus terreus Thom AUMC 13019	Biomass	-	-	-	-
	CS	-	-	-	-
Cladosporium cladosporioides (Fresenius) de Varies AUMC 13021	Biomass	-	-	-	-
	CS		--	-	-
Corynoas cussepedonium (Emmons) Von Arx AUMC 13016	Biomass	-	-	-	-
	CS	-	-	-	
Eupenicillium hirayamae Sott and Stolk AUMC 13009	Biomass	-	-	-	-
	CS	-	-	-	-
Emericella nidulans (Eidam) Vuillemin AUMC 8623	Biomass	-	-	-	-
	CS	+	0.50	325	Broad
Fusarium subglutinans (wollenweber and Reinking) Nelson *et al.* AUMC 13008	Biomass	-	-	-	-
	CS	-	-	-	-
Humicolagrisea Traaen AUMC 13020	Biomass	-	-	-	-
	CS	-	-	-	-
Penicillium aurantiogriseum Dierckx AUMC 13013	Biomass	-	-	-	-
	CS	-	-	-	-
Penicillium brevicompactum Dierckx AUMC 13014	Biomass	-	-	-	-
	CS	-	-	-	-
Penicillium pinophilum Hedgcock AUMC 13011	Biomass	-	-	-	-
	CS	+	0.59	360	Sharp
Scedosporium apiospermum (Sacc.) Sacc. AUMC 13017	Biomass	-	-	-	-
	CS	-	-	-	-

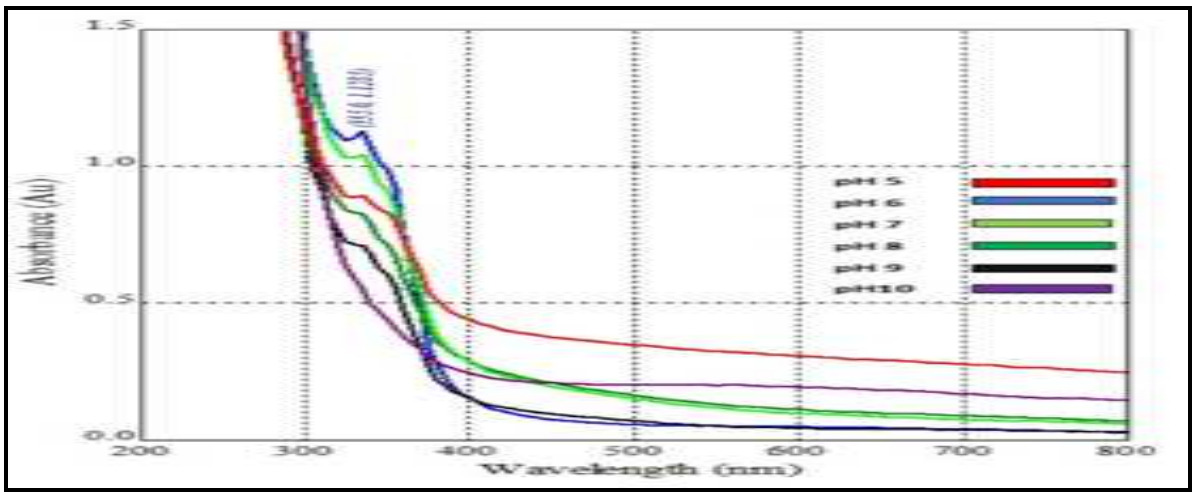

Figure No.1: Biosynthesis of CuO NPs by *A. fumigatus* biomass as influenced by different pH values

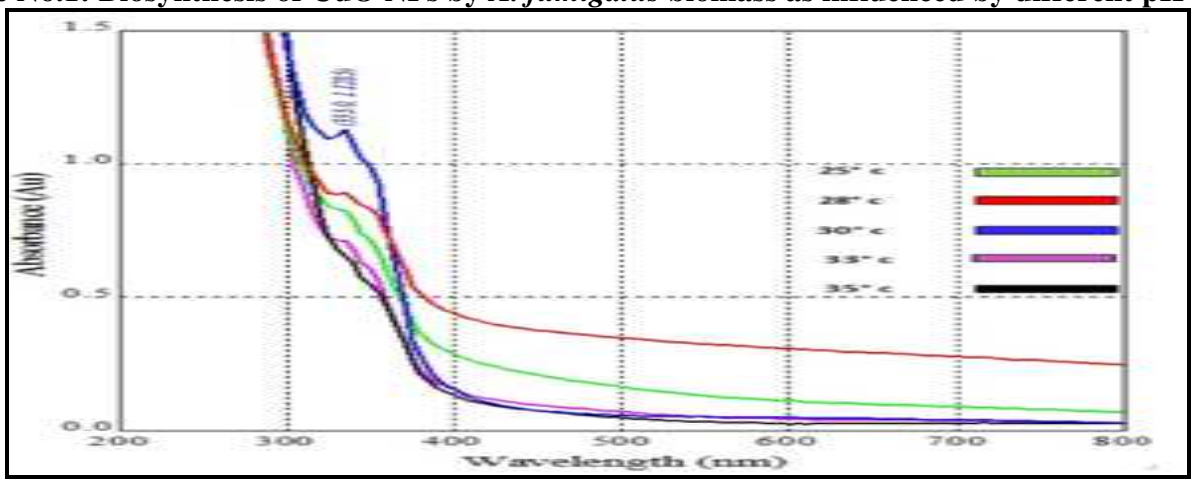

Figure No.2: Biosynthesis of CuO NPs by *A. fumigatus* biomass as influenced by different reaction temperatures

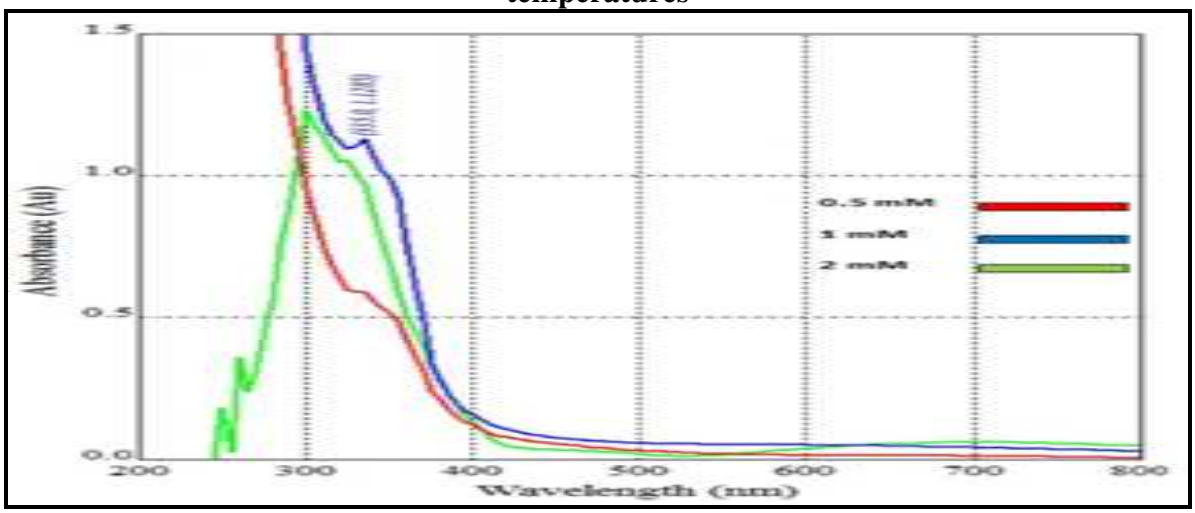

Figure No.3: Influence of changing copper nitrate concentration on the biosynthesis of CuO NPs using *A. fumigatus* biomass

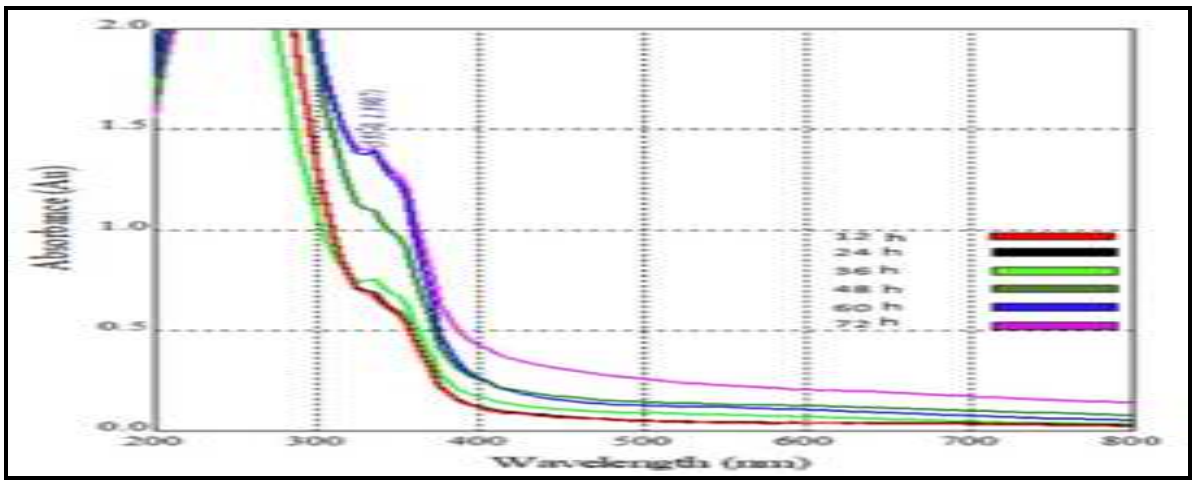

Figure No.4: Biosynthesis of CuO NPs using *A. fumigatus* biomass after different reaction times

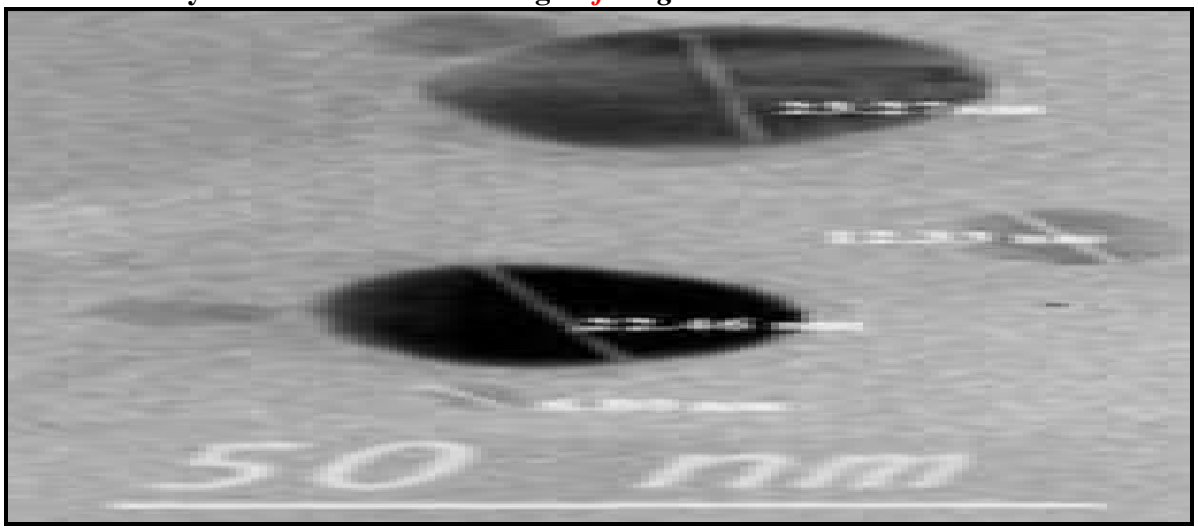

Figure No.5: TEM micrograph of CuO NPs biosynthesized using *A. fumigatus* preformed biomass from copper nitrate

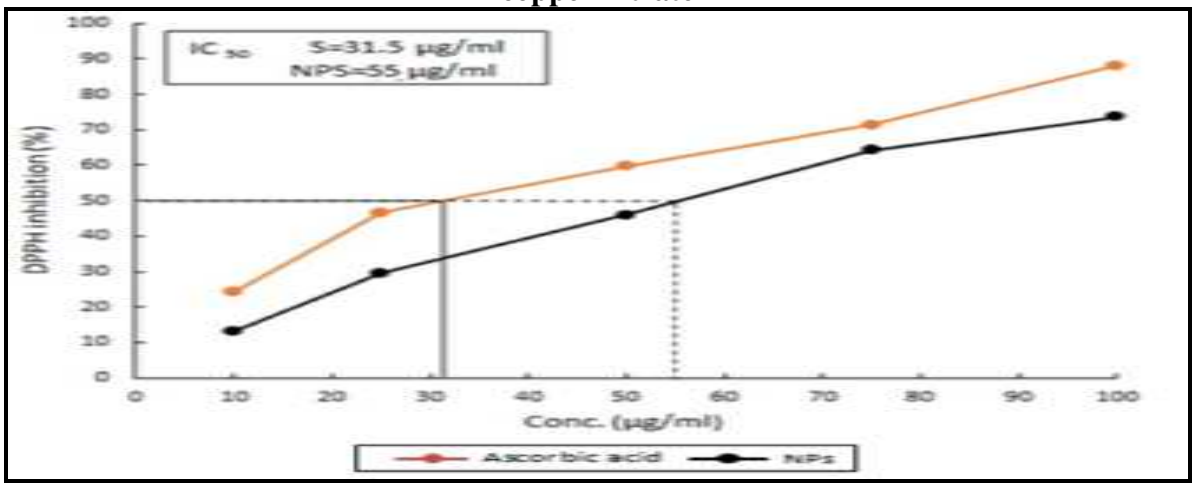

Figure No.6: DPPH free radical scavenging activity of the biosynthesized CuO NPs in comparison with ascorbic acid

CONCLUSION

Biosynthesis of copper oxide nanoparticles is rarely explored using fungi. This work is a trial to bridge the gap in this respect. *Aspergillus fumigatus* was suggested in a preliminary screening of nineteen different fungi as good producer for these nanoparticles. This fungus was isolated from soil sample collected near Giza, Egypt. Most important factors affecting the reaction were optimized for this fungus. The absorption spectrum of the biosynthesized nanoparticles reached its maximum at 335nm with an intense peak. These nanoparticles were spherical in shape and had good free radical scavenging activity.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Roxy 11757, Cairo, Egypt for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Senapati S, Ahmad A, Khan M I, Sastry M, Kumar R. Extracellular biosynthesis of bimetallic Au-Ag alloy nanoparticles, *Small*, 1(5), 2005, 517-520.
2. Ghorbani H R, Fazeli I, Asghar A. Biosynthesis of copper oxide nanoparticles using extract of *E.coli*, *Oriental J Chem,* 31(1), 2015, 515-517.
3. El-Sawaifi S F, Palmieri J R, Hockey K S, Rzigalinski B A. Antioxidant nanoparticles for control of infectious disease, *Infect Disord Drug Targets*, 9(4), 2009, 445-452.
4. Braugghler J M, Duncan C A, Chase L R. The involvement of iron in lipid peroxidation: importance of ferrous to ferric ratio in initiation, *J BiolChem*, 61(22), 1986, 102-182.
5. Umber A, Naveed S, Ramzan N. Selection of a suitable method for the synthesis of copper nanoparticles, *NANO: Brief reports and reviews*, 7(5), 2012, 1230005.
6. Ayoman E, Hossini G, Haghighi, N. Synthesis of CuO nanoparticles and study on their caalytic properties, *International J Nanosci Nanotechnol,* 11(2), 2015, 63-70.
7. Khashan K S, Sulaiman G M, Abdulameer F A. Synthesis and antibacterial activity of CuO nanoparticles suspension induced by laser ablation in liquid, *Arab J SciEng*, 41(1), 2016, 301-310.
8. Hayashi H andakuta Y. Hydrothermal synthesis of metal oxide nanoparticles in supercritical water, *Materials,* 3(7), 2010, 3794-3817.
9. Kayani Z N, Umer M, Riaz S, Naseem S. Characterization of copper oxide nanoparticles fabricated by the sol-gel method, *JElectron Mater*, 44(10), 2015, 3704-3709.
10. Khan S T, Musarrat J, Al-Khedhairy A A. Countering drug resistance, infectious diseases, and sepsis using metal and metal oxides nanoparticles: current status, *Colloids Surf B*, 146, 2016, 70-83.
11. Karimi J and Mohsenzadeh S. Rapid, green, and eco-Friendly biosynthesis of copper nanoparticles using flower extract of *Aloe Vera*. *Synthetic and Reactivity in Inorganic, Metal-organic, and Nano-metal Chem*, 45(5), 2015, 895-898.
12. Vanathi P, Rajiv P Sivaraj R. Synthesis and characterization of *Eichhornia*-mediated copper oxide nanoparticles and assessing their antifungal activity against plant pathogens, *Bull Mat Sci*, 39(5), 2016, 1165-1170.
13. Ijaz F, Shahid S, Khan S A, Ahmad W, Zaman S. Green synthesis of copper oxide nanoparticles using *Abutilon indicum* leaf extract: Antimicrobial, antioxidant and photocatalytic dye degradation activities, *Tropical J Pharm Res*, 16(4), 2017, 743-753.

14. Yugandhar P, Thirumalanadhuni Vasavi T, Uma P, Maheswari Devi M, Savithramma N. Bioinspired green synthesis of copper oxide nanoparticles from *Syzygiumalternifolium* (Wt.) Walp: characterization and evaluation of its synergistic antimicrobial and anticancer activity, *Appl Nanosci*, 7(7), 2017, 417-427.
15. Abboud Y, Saffaj T, Chagraoui A, El-Bouari A, Brouzi K, Tanane O, Ihssane B. Biosynthesis, characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract (*Bifurcariabifurcata), Appl Nanosci*, 4(5), 2014, 571-576.
16. Honary S, Barabadi H, Gharael-Fathabad E, Naghibi F. Green synthesis of copper oxide nanoparticles using *Penicillium aurantogriseum, Penicillium citrinum and Penicillium waksmanii, Digest J Nanomat Biostruct*, 7(3), 2012, 999-1005.
17. Cuevas R, Durán N, Diez M C, Tortella G R, Rubilar O. Extracellular biosynthesis of copper and copper oxide nanoparticles by *Stereumhirsutum*, a native white-rot fungus from Chilean forests, *J Nanoma*, Article ID 789089, 2015, 1-7.
18. Mandal D, Bolander M E, Mukhopadhyay D, Sarkar G, Mukherjee P. The use of microorganisms for the formation of metal nanoparticles and their application, *Appl Microbiol Biotechnol*, 69(5), 2006, 485-492.
19. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, *J Agric Food Chem*, 40(6), 1992, 945-948.
20. Rehana D, Mahendiran D, Kumar R S, Rahiman A K. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts, *Biomed Pharmacother,* 89, 2017, 1067-1077.
21. Phull A, Abbas Q, Ali A, Raza H, kim S J, Zia M and Haq I. Antioxidant, cytotoxic and antimicrobial activities of green synthesized silver nanoparticles from crude extract of *Bergenia ciliate*, *Future J Pharma Sci*, 2(1), 2016, 31-36.

Please cite this article in press as: Mohamed Ghareib *et al.* Green biosynthesis of copper oxide nanoparticles using some fungi isolated from the egyptian soil, *International Journal* of *Research in Pharmaceutical and Nano Sciences,* 7(4), 2018, 119-128.