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Research Paper

Biosynthesis of metallic nanoparticles using fungi and their applications

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ABSTRACT

Biosynthesis of different metallic nanoparticles (NPs) using different fungal species is one of the most essential areas in the field of nanobiotechnology as an eco-friendly process. The present study demonstrated the biosynthesis of certain metallic nanoparticles (silver, copper and aluminum) using three fungal isolates *Alternaria alternata*, *Penicillium duclauxii* and *Aspergillus niger*, respectively. The biosynthesized NPs were characterized using Ultraviolet-Visible Spectroscopy, Transmission electron microscopy, Dynamic light scattering using Zeta potential and X-ray diffraction. The optimum experimental conditions for the biosynthesis of these metallic nanoparticles were found to be agitation speed of 150 rpm, a temperature of 28°C, a substrate concentration of 10 mM, weight of used mycelium was 10 g and a pH of 6.0. The wear test of the lubricating grease using these NPs, as additives, showed a significant reduction of the wear loss as compared with blank. In this respect, the Cu-NPs showed the most effective reduction of wear rate. The Scanning electron microscopy micrographs and Energy dispersive X-ray chemical analysis confirmed the formation of a tribo-chemical film comprising elements from the biosynthesized nanoparticles under investigation. Also, the biosynthesized NPs were used as photo-catalysts to increase the percentage of photo degradation of P-nitrophenol (PNP), as an example of toxic aromatic compounds. The obtained results confirmed that, the myco-synthesized Ag, Cu and Al nanoparticles can offer a promising eco-friendly and an alternative way to the chemical and synthetic methods, and could have wide applications in different fields.

Key words: Myco-nanotechnology, nanoparticles, zeta potential, tribology, photocatalysts.

Abbreviation: AgNPs: Silver nanoparticles; **AlNPs:** Aluminum nanoparticles; **ANOVA:** One- way analysis of variance; **C:** Concentration of PNP at time (t); **Co:** Intial concentration of PNP; **Cu-NPs:** Copper nanoparticles; **Dox (Cz):** Dox (Czapex) Agar medium; **DLS:** Dynamic light scattering; **EDX:** Energy dispersive X-ray; **EP:** Extreme pressure; **EPRI:** Egyptian Petroleum Research Institute; **fcc:** Face centered cubic; **HRC:** Hardness; **JCPDS:** Joint Committee on Powder Diffraction Standards; **L:** Pin with lubricating grease only (control); **L1:** Pin with lubricating grease + AgNPs; **L2:** Pin with lubricating grease + Cu-NPs; **L3:** Pin with lubricating grease + AlNPs; **MEA:** Malt extract agar medium; **mM:** Milli molar; **mv:** Milli volt; **nm:** Nanometer (10^{-9}); **NPs:** Nanoparticles; **PNP:** Para-nitro phenol; **ppm:** Part per million; **RCMB:** Regional Center of Mycology and Biotechnology; **rpm:** Round per minute; **SEM:** Scanning electron microscopy; **TEM:** Transmission electron microscopy; **UV-vis:** Ultra violet visible spectroscopy; **XRD:** X-ray diffraction; **θ:** Theta.

INTRODUCTION

Nanotechnology is the science that deals with the matters in the size range of 1 to 100 nm; these matters are called nanoparticles (NPs). Nanoparticles acquire special properties different from their properties in bulk size (Mansoori, 2005). It is important to synthesize NPs using safe protocols. The target of nanobiotechnology has many benefits such as feasibility, can be scaled up, covering large areas by growth of mycelia, etc. (Mansoori, 2005).

Many microorganisms are used to synthesize inorganic nanoparticles either intracellulary or extracellulary, so it can be used as environmentally safe nanofactroies (Mohanpuria et al., 2008).

Myco-nanotechnology is a new term that is defined as the fabrication of NPs by fungi and their subsequent applications (Jebali et al., 2011). There were other benefits of using fungi in biosynthesis of metallic NPs such as ease to scale up and cultivate, high wall binding capacity and high tolerance towards metals (Zeinab et al., 2011). For example, the yeast *Trichosporon jirovecii* could tolerate the cadmium toxicity by its conversion into CdS NPs as a detoxification defense mechanism using cysteine- desulfurase enzyme (El-Baz et al., 2016). In the process of NPs biosynthesis using fungus, the fungal mycelium is mixed with metal salt in the form of solution, the fungus produce enzymes and bioactive substances to reduce toxic metal ions to non-toxic metallic NPs (Vahabi et al., 2011).

To study the special characteristics of the produced NPs, different techniques, such as ultraviolet-visible spectroscopy, transmission electron microscopy (TEM), dynamic light scattering (DLS) using Zeta potential and X-ray diffraction (XRD) techniques, were used (Guangquan et al., 2012).

Optimization experiments were carried out to determine the best parameters that contributed in the ideal production of different nanoparticles using fungi, these parameters were: metal salts, concentrations, mixing ratio of filtrate to silver nitrate, temperature and pH (Sharma, et al., 2009).

Because of the special unique properties of metallic NPs, they emerge in vital applications in different fields such as engineering, medical, optical, catalysis, etc. (Chattopadhyay and Patel, 2012). In engineering of machines and tribology, different synthesized nanoparticles can be added to lubricating oils and greases to increase the efficiency of machines through decreasing wear, tolerate high pressure, etc. (Li et al., 2006).

Organic contaminants such as benzidine, naphthalene, PNP and other aromatic compounds have been presented into the environment and ground waters as sources of pollution that have to be removed using photocatalysts. Addition of NPs as silver has improved the photocatalytic activity of semiconductor (Linsebigler et al., 1995).

The overall objective of the present study was the myco-synthesis of very small size extracellular nanoparticles using the fungal isolates. The specific objectives were to characterize NPs using UV, TEM, DLS and XRD, and investigate the best parameters that contribute to the ideal production of different nanoparticles. Moreover, the determination of their applications in various fields was done.

MATERIALS AND METHODS

In this study, different soil samples were used for isolation of fungi which were collected from two gardens (The Regional Center for Mycology and Biotechnology" RCMB", Al-Azhar University and Faculty of Science, Ain-shams University, Cairo, Egypt). The identification of the fungal isolates was carried out on the basis of growth and microscopic morphology using Image Analyzer System (Version Pro .3) and the universal manuals (John, 1979; De Hoog and Guarro, 1995; Kendrick, 2000) in RCMB.

The salts: silver nitrate $AgNO_3$, copper sulfate $CuSO_4$ and aluminum sulfate $Al_2 (SO_4)_3$ were used for biosynthesizing NPs. Lithium lubricating grease as described elsewhere (El-Adly, 2004) was used as a base of lubricant and obtained from the Egyptian Petroleum Research Institute (EPRI), Cairo, Egypt. It was used to evaluate the AgNPs, Cu-NPs and AlNPs as lubricant additives. The tested dose of each NP (0.3 wt. %) was added to lubricating grease.

Biosynthesis of nanoparticles using fungal isolates

The fungal isolates were inoculated into the flasks containing 50 ml of malt extract broth media and incubated at 25 - 28°C and 150 rpm (Kowshik et al., 2002). After 7 days of incubation, each growing mycelium was separated using Whatmann filter paper. Ten grams of it was added to 100 ml of deionized water and incubated in the shacking incubator at 150 rpm, 28°C for 3days to produce enzymes and metabolites (Durán et al., 2005). At the end of incubation period, 10 mM of each metal salt was added to the fungal filterate to produce the nanoparticles.

Characterization of myco-synthesized nanoparticles

UV-visible spectroscopy analysis

Detection of NPs was primarily observed visually by changing the color of the cell filtrate after treatment with each metal salt. Characterization of the synthesized NPs was further carried out using the UV-visible spectrophotometer, in RCMB. Characterization of the synthesized Ag-NPs was carried out using scanning absorbance spectrum ranging from 240 to740 nm (Vigneshwaran et al., 2007). It was 448 nm when excited at 433 nm case of copper oxide nanoparticles (Honary et al., 2012) and for aluminum ion was ranged from 400 to 700nm (Tao et al., 2010).

Transmission electron microscopy (TEM)

The shape and particle size of nanoparticles were observed by TEM (JEOL JEM 1010 instrument studies), at an accelerating voltage of 70 Kev in RCMB (Basavaraja et al., 2008).

Dynamic light scattering (DLS) using zeta potential technique

Size distribution, average size and stability of the synthesized nanoparticles were determined by DLS

(Malvern Zeta sizer Ver. 6.32, MAL 10171664), in the Central lab of (EPRI) (Clogston and Patri, 2008).

X-ray diffraction (XRD)

X-ray diffraction pattern analysis was carried out in the central lab of EPRI using (X-ray 7000 Shimadzu, Japan), to know the face center cubic crystalline (fcc) natures of the myco-synthesized nanoparticles **(**Cullity, 1978; Lei and Fan, 2006).

Assessment the biosynthesis of nanoparticles under different physical factors

Effect of agitation/ static conditions on nanoparticles biosynthesis

In this experiment, the agitation (shaking) speeds; 150, 200 rpm and static condition were used for cultivation of fungal isolates in 50ml DOX (Cz) broth medium, to study the effect of different agitation speeds on biosynthesis of Ag, Cu, and Al NPs during the incubation, at 28°C, for 7days **(**Vahabi et al., 2011).

Effect of temperature

Assessment the biosynthesis of mycosynthesized nanoparticles at different incubation temperatures (20, 28 and 37°C) (Vahabi et al., 2011).

Effect of metal salt concentration (substrate concentration)

In this experiment, two different concentrations of metal salts "1 and 10 mM" were used. Other parameters of the experiment were kept constant (Mehra and Winge, 1991).

Effect of mycelium weight (size of inoculum)

During the myco-synthesis, 5 and 10 g of each fungal mycelium were tested to study the effect of mycelium weight on NPs production (Vahabi et al., 2011).

Effect of pH

The pH of the cultural mixture was adjusted to (4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) using 1M HNO_3 and 1M NaOH solutions. This study was done to obtain the optimum conditions for maximum biosynthesis of elemental nanoparticles (Sneha et al., 2010).

Statistical analysis

Statistical analysis of experimental data was performed using SPSS program v. 20; to detect the optimum physical factors for the biosynthesis of NPs. The differences among treatments in each experiment were compared using one-way analysis of variance (ANOVA) followed by post- hoc test. Except for the experiments which tested the effect of concentration of metal salts and mycelium weight, they were compared using two samples t-test. The probability of error (P value) at 0.05 or less was considered significant, while at 0.01 and 0.001 was considered highly significant (Zar, 1999).

Applications of nanoparticles

Anti-wear test procedure and evaluation

The anti-wear properties of the lithium lubricating grease with and without biosynthesized Ag, Cu and AlNPs were determined using pin on disc apparatus (El-Adly et al., 2015). The tested specimens (Pin) were AISI1023 steel with diameter of 10 mm and HRC about 20.5. However, the disc was manufactured from wear resisting steel (Hardox 600) with diameter of 10 cm and HRC of about 59.5. Pin-on-disc experiments lasted for 3 h with agitation speed of 300 rpm, and load of 150 N, using the lithium grease with and without NPs. The weight losses in pin were calculated to evaluate the efficiency of prepared NPs as anti-wear additives. However, the morphology of the obtained metal surfaces was investigated by SEM "Scanning electron microscope" model (JEOL JSM – 6510) supplied with EDX "Energy dispersive X-ray" as elemental analysis system (EAS) in (EPRI).

Photodegradation of p-nitrophenol (PNP) using nanoparticles

In this study, we evaluated the photoactivity of the prepared NPs by photodegradation of P-nitrophenol and also determined the effect of Ag as a promoter on the activity of Cu loaded on AlNPs as a support. After the myco-synthesis of NPs, they were physically mixed together at a ratio of 2:1:2 for Ag, Cu and Al, respectively by dispersion in the solvent (water) and then sonicated for 1 h. Thereafter, the mixture was dried at 100°C for 24 h. The photocatalyst (0.01 g) was suspended in 100 ml of PNP in water solution (40 ppm). Air was bubbled into the suspension to maintain constant oxygen saturation in the solution. For adsorption-desorption equilibrium, the suspension was magnetically stirred for 40 min in the dark. The solution was then exposed to visible-light irradiation for 12 h and 5 ml of the suspension drawn at specific time intervals. The degradation was monitored by measuring the absorbance

at wavelength ranging from 240 to 740 nm using a double beam UV–vis spectrophotometer (Abdul et al., 2016).

RESULTS AND DISCUSSION

Identification of fungal isolates

The identification of the fungal isolates was carried out on the basis of macroscopic and microscopic morphology, using the universal manuals (John, 1979; De Hoog and Guarro, 1995; Kendrick, 2000) as follows:

Fungal isolate no. 1

Conidiophores were mostly unbranched, while conidia were ellipsoidal with a short cylindrical beak and rug lose with muriform septation. On the basis of the previously mentioned microscopic morphology, the fungal isolate was identified as *Alternaria alternata* by the aid of the following universal manuals (John, 1979; De Hoog and Guarro, 1995; Kendrick, 2000).

Fungal isolate no. 2

The conidiophores are borne with smooth walls to finely roughened, terminating in closely packed metulae and sometimes inflated apically; phialides acerose, conidia ellipsoidal to apiculate with smooth walls to finely roughened, borne in chains. On the basis of the previously mentioned microscopic morphology, the fungal isolate was identified as *Penicillium duclauxii* by the aid of the following universal manuals (John, 1979; De Hoog and Guarro, 1995; Kendrick, 2000).

Fungal isolate no. 3

The conidiophores were smooth-walled and hyaline and end with sub-spherical vesicles. Conidiogenous cells were biseriate. Metulae was twice as long as the phialides, while the conidial heads are radiate. On the basis of the previously mentioned microscopic morphology, the fungal isolate was identified as *Aspergillus niger* by the aid of the following universal manuals (John, 1979; De Hoog and Guarro, 1995; Kendrick, 2000).

Biosynthesis of nanoparticles using fungi

Biosynthesis of AgNPs using A. alternata

The crude cell filtrate of A. alternata was changed from light yellow to brown in a few hours after the addition of $AgNO_3$ solution, while no color change was observed in the filtrate without $AgNO_3$. This clearly indicated the formation of AgNPs after 24 h. Wiley et al. (2006) confirmed that the brown color produced after the addition of $AgNO_3$ was referred to the AgNPs production (Wiley et al., 2006).

Biosynthesis of Cu-NPs using P. duclauxii

The crude cell filtrate of P. duclauxii containing CuSO4 solution changed from blue to dark green, indicating the formation of Cu-NPs, after 48 h. This result coincide with that of Yu et al. (2011) who reported that when the filtrate of P. fluorescens was mixed with $CuSO_4$ solution and cultured for 48 h, the color of the mixture changed to dark green due to the production of Cu-NPs (Yu et al., 2011).

Biosynthesis of AlNPs using A. niger

The mixing of $Al_2(SO_4)_3$ with the crude cell filtrate of A. niger led to the formation of AlNPs with yellowish brown color. Farahmandjou and Golabiyan (2015) recognized the color of Al_2O_3 NPs that was produced by combustion method to brown (Farahmandjou and Golabiyan, 2015).

Characterization of nanoparticles

Uv-visible spectroscopy analysis

The stability of the synthesized metallic NPs was assessed by monitoring the UV-visible analysis of the reaction mixture after different incubation times. The maximum absorbance value, which corresponds to SPR "Surface Plasmon Resonance" of the NPs was observed and increased by increasing the reaction time. The maximum absorbance was obtained at wavelength of 440 nm after incubation time of 24 h in the case of AgNPs, while in case of Cu-NPs, it was 450 nm after 48 h, and 450 nm in the case of AlNPs after incubation for 72 h. The results were in line with the report of Vigneshwaran et al. (2007) indicating that when fungal filtrates are mixed with $AgNO_3$, AgNPs was produced at maximum absorbance at “436 nm". According to the report of Honary et al. (2012), copper oxide nanoparticles' appear as broad emission peak at 448 nm, but for the synthesized aluminum oxide (Al_2O_3) NPs, it was near "475 nm" (Bhattacharya et al., 2004).

Transmission electron microscopy (TEM)

The synthesized AgNPs are spherical, polydispersed and ranged in size from 5 to 13 nm (Figure 1A). Also, Vahabi et al. (2011) reported that the size of biosynthesized AgNPs ranged in size from 5 to 50 nm (Vahabi et al., 2011). In the case of Cu-NPs, it ranged from 37 to 67 nm with the

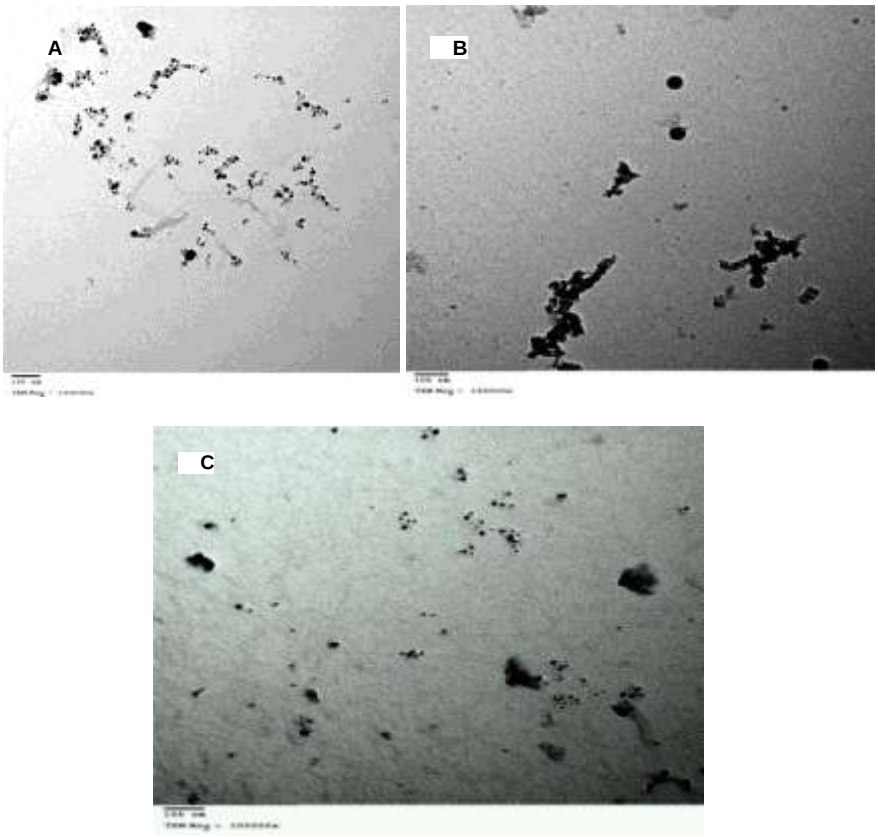

Figure 1: (A) TEM of the biosynthesized Ag-NPs, (B) TEM of the biosynthesized Cu-NPs and (C) TEM of the biosynthesized Al-NPs.

presence of spherical and hexagonal shapes (Figure 1B). In addition, this result is in line with the results of Chattopadhyay and Patel (2012) who recognized that the freshly prepared Cu-NPs were about 70 nm for most particles, while AlNPs average diameter was about 10 nm with sphere-like structure (Figure 1C) (Farahmandjou and Golabiyan, 2015).

Dynamic light scattering (DLS) using Zeta potential technique

In this technique, each nanoparticle was measured twice (the first time in fresh state and the second time after 2 months from biosynthesis) to confirm the stability of the myco-synthesized nanoparticles. Particle surface characteristics and charge played an important role in the particle's physical state, stability in different media, agglomeration tendencies and interaction with biological systems (Clogston and Patri, 2008). In the case of fresh AgNPs, the Zeta potential was -21.7 mv, while, it was -18.3 mv after 2 months from biosynthesis. Similarly, in the case of freshly prepared Cu-NPs, the Zeta potential was -24.6 mv, while that biosynthesized from 2 months, the Zeta potential was -21.5 mv. However, the zeta potential of AlNPs was -27.5 mv and after 2 months from biosynthesis, it was -25.5 mv. These results confirmed that the myco-synthesized NPs were still stable and active because the Zeta potential decreased in small degrees in both states" fresh and after 2 months from biosynthesis" and has the same "negative charge".

X-ray diffraction

The prepared nanoparticles were analyzed using XRD. There were four well-defined characteristic peaks at 38.27, 44.55, 64.29 and 77.8o, according to (fcc) structure of silver (Figure 2) (Ref. Code no. 01-087-0719). This result is in line with the study of Acharya and Mohanta (2017) who reported that the peaks at 2 theta equal 38.0, 45.02 and 62.65o corresponded to AgNPs (JCPDS file No. 03-0921).

The relatively sharp peaks confirmed the crystalline nature of AgNPs. The XRD patterns of CuO-NPs showed four distinct peaks at 31.60, 35.25, 38.39 and 48.58o, which were well indexed to the cubic CuO (Figure 3) (Ref. Code no. 00-048-1548). Similar results were observed by Phiwdang et al. (2013) who reported that the characteristic peaks located at 32.58, 35.47, 38.97 and 48.74o confirmed the crystalline nature of CuO nanoparticles, respectively

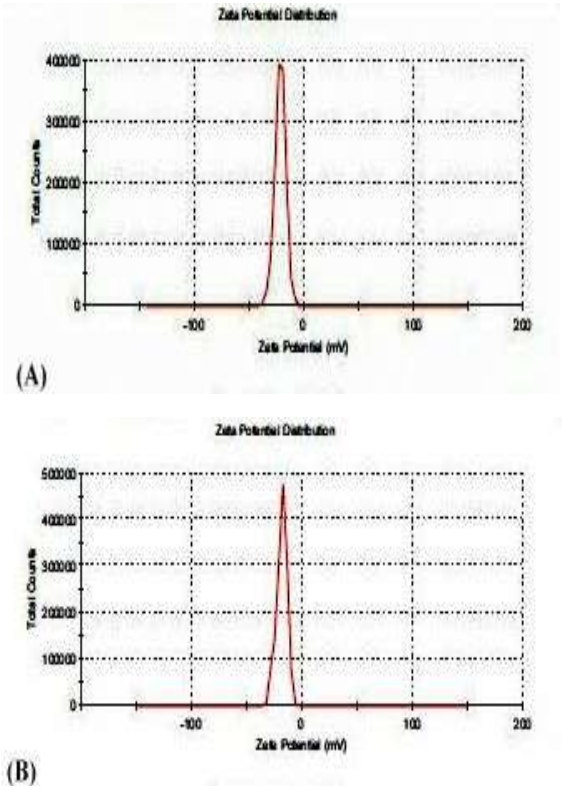

Figure 2: (A) DLS of fresh biosynthesized silver nanoparticles, (B) DLS of biosynthesized silver nanoparticles "after 2months of biosynthesis" using Zeta potential.

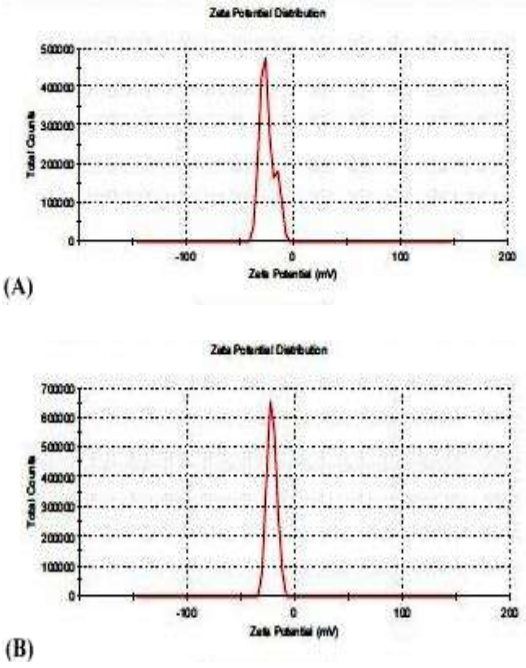

Figure 3: (A) DLS of fresh biosynthesized copper nanoparticles, (B) DLS of biosynthesized copper nanoparticles "after 2months of biosynthesis" using Zeta potential.

based on (JCPDS 80-1268) (Phiwdang et al., 2013). Furthermore, the formation of Al_2O_3 nanoparticles was primarily confirmed by XRD pattern and four peaks appeared at 25.3, 35.23, 43.56 and 66.7o with (Ref. Code no.00-001-1243). These peaks were compared with peaks of nano sized Al_2O_3 nanoparticles prepared by the sol-gel technology and were in line with four of the JCPDS file (71-1683) (Dhawale et al., 2018).

Assessment the biosynthesis of nanoparticles under different physical factors

The effect of agitation/ static conditions on NPs biosynthesis

From the One-way ANOVA of normal data carried out it was declared that there was a significant difference in the biosynthesis of silver, copper and aluminum nanoparticles under different agitation speeds. Post- hoc test proved that 150 rpm was the optimum agitation speed for the biosynthesis of Ag, Cu and Al nanoparticles using A. alternata, P. duclauxii and A. niger, respectively with 0.000 level of significance (P= 0.000). This result was in line with the study of Vahabi et al. (2011) who reported on the synthesis of NPs and showed that the fungus have to be grown under continuous mixing condition by a magnetic stirrer at 150 rpm for 72 h (Vahabi et al., 2011). In addition, Mehra and Winge (1991) reported that certain fungi have metabolites as agents for their own survival when exposed to environmental stresses like toxic materials (for example, metallic ions), predators, shaking and temperature variations.

The effect of temperature

One-way ANOVA of normal data was carried out revealing that there was a significant difference in the biosynthesis of Ag, Cu and AlNPs when tested at different incubation temperatures. Post-hoc test proved that 28°C was the optimum temperature for the biosynthesis of NPs with 0.000 level of significance (P= 0.000). This result is in line with the study of Vahabi et al. (2011) who reported that the optimum fungal growth temperature ranged from 25 to 28°C to synthesize nanoparticles.

The effect of concentration of metal salt (Substrate concentration)

Two samples t-test was carried out and revealed that there was a significant difference in the biosynthesis of elemental nanoparticles upon changing the concentration of metal salt with 0.000 level of significance (P= 0.000). The test also declared that 10 mM was the best concentration of metal salts for the biosynthesis of Ag, Cu and Al nanoparticles using A. alternata, P. duclauxii and A. niger, respectively. This result is in line with the study of Guangquan et al. (2012) who reported that AgNPs were synthesized using 50 ml cell filtrate of A. terrus mixed with 10 mM of $AgNO_3$ solution (Guangquan et al., 2012).

The effect of mycelium weight

From the two samples t-test carried out it was declared that there was a significant difference in the biosynthesis of the elemental nanoparticles upon using different mycelial weight with 0.000 level of significance (P= 0.000). The test declared that 10 g was the optimum mycelium weight used for the biosynthesis of Ag, Cu and Al nanoparticles using A. alternata, P. duclauxii and A. niger, respectively. This result is in line with the result of Vahabi et al. (2011) who reported that in a typical biosynthesis production scheme of AgNPs, 10 g of Trichoderma reesei fungus wet biomass was mixed with a 100 ml aqueous solution of 1 mM $AgNO_3$. Table 1 shows all the results of the previous parameters of the biosynthesis of NPs.

The effect of pH

From the One-way ANOVA of normal data carried out it was revealed that there was a significant difference in the biosynthesis of elemental nanoparticles at different pH values with 0.000 level of significance (P= 0.000). Post-hoc test proved that pH 6.0 was the optimum pH value for the biosynthesis of Ag, Cu and Al nanoparticles using A. alternata, P. duclauxii and A. niger, respectively with level of significance equal or less than 0.05 (P</= 0.05) (Table 2). Also, Manjunath and Padma (2012) reported that the optimum experimental conditions for biosynthesis of AgNPs by A. niger were found to be at a temperature 37°C, pH of 6.0 and a substrate concentration of 2.0 mM.

Applications of nanoparticles

Wear behavior of lubricating grease with NPs as additives

The essential property of lubricating grease has to possess the ability to form a thin film between rubbing surfaces in order to be able to prevent metal to metal friction. This property, as well as, different other characteristics is mainly determined by the type of additives or improvers. The anti-wear properties of the lithium lubricating grease with and without biosynthesized NPs were evaluated. Table 3 showed the variation of the wear loss after addition of NPs to lubricating grease, as nano-additives. It can be seen that the anti-wear and friction reduction properties of

Table 1: Biosynthesis of nanoparticles under different physical factors (Agitation speeds, temperatures, substrate concentrations and mycelial weight).

NPs (mg)	Parameter									
	Agitation speed			Temperature			Substrate conc.		Mycelium weight	
	Static	150(rpm)	200(rpm)	20(°C)	28(°C)	37(°C)	1(mM)	10(mM)	5(gm)	10(gm)
Silver nanoparticles	119.67 ± 0.882	**491.67 ± 1.202**	255.33 ± 0.333	216 ±0.577	**430.33 ± 0.882**	150.67 ± 1.202	282.67 ± 1.453	**432 ± 1.732**	0	**315.33 ± 2.603**
Copper nanoparticles	74 ± 2.082	**351.67 ±2.186**	162 ± 1.764	171± 2.082	**276 ± 2.082**	0	176.67 ± 3.528	**343.33 ± 3.283**	89.33 ± 2.333	**316 ± 3.055**
Aluminium nanoparticles	81 ± 2.082	**93.33 ± 2.028**	41 ± 2.082	0	**93 ± 2.082**	50 ± 2.887	57.67 ± 4.333	**90.67 ± 2.963**	71 ± 2.082	**93.33 ± 4.410**

Table 2: Biosynthesis of nanoparticles under different pH values.

NPs (mg)	pH					
	4.0	5.0	6.0	7.0	8.0	9.0
Silver nanoparticles	442.33±1.453	205.33±0.882	476.33±0.882	443± 1.528	215.67± 1.202	180.67±15.344
Copper nanoparticles	175± 2.646	165± 2.887	341± 2.082	91± 3.786	71.33± 3.480	62.33± 3.383
Aluminium nanoparticles	64± 6.083	82.33± 4.333	93.33± 2.404	44± 3.215	87.33± 2.404	0

(±) = standard error between the three replicates.

Table 3: Assessment of the pin weight before and after wear test.

Treatments	Weight of pin before test (g)	Weight of pin after test (g)	Weight loss (g)
Grease with AgNPs	15.5430	15.5290	0.014
Grease with Cu-NPs	15.3397	15.3277	0.012
Grease with AlNPs	14.7480	14.7047	0.023
Lithium grease only	15.55	14.95	0.60
Metal pin only (Blank)	15.60	14.65	0.95

lubricating grease were improved by the addition of NPs. As shown in Table 3, the weight loss in the case of the blank pin (metal with metal) was observed to be the highest due to high friction, while in the case of the pin using lubricating grease (L); the friction decreased and the weight loss was lower. However, using NPs as nano-additives to lubricating grease led to a decreased weight loss. In the case of using AlNPs (L_3), the weight loss was higher than Cu-NPs (L_2) and Ag-NPs (L_1); however, the Cu-NPs were the lowest. This means that the use of Cu-NPs as nano-additives is the best choice to reduce both friction and the wear behavior.

These results were confirmed by the morphological characteristics and chemical constituents of the worn surfaces on the steel pin using SEM and EDX (Figures 4 to 8). These figures showed that there are many deep pits and large area exfoliation on the worn surface in the case of blank indicating that severe adhesive wear had occurred, while the pin surfaces lubricated in cases of L, L_1, L_2 and L_3 were smooth. In particular in the case of L_2 (grease with Cu-NPs), the pin surface was the smoothest as compared with L, L_1 and L_3.

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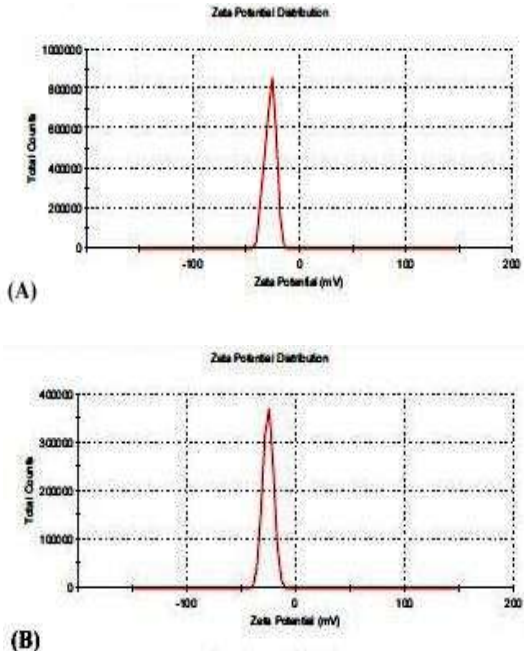

Figure 4: (A) DLS of freshly prepared aluminum nanoparticles, (B) DLS of mycosynthesized aluminum nanoparticles "after 2months of biosynthesis" using Zeta potential.

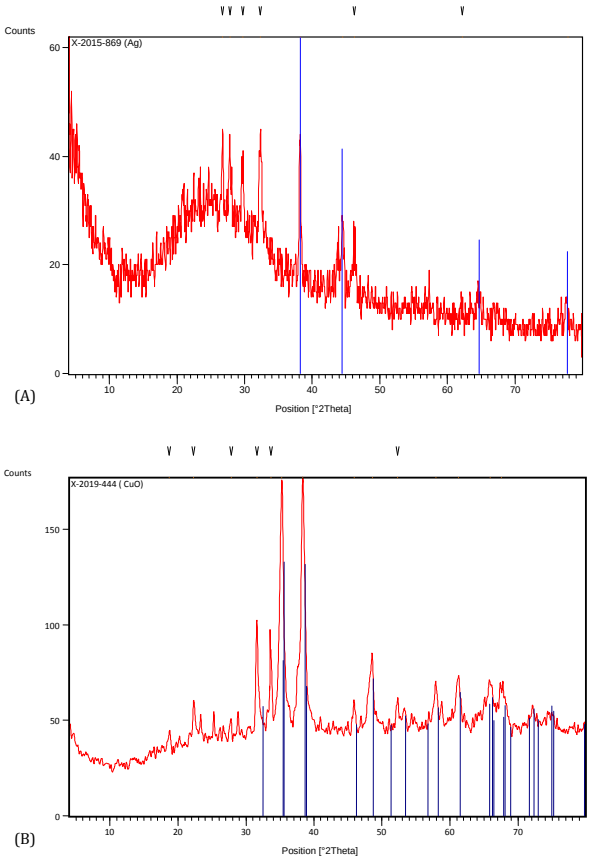

Figure 5: (A) XRD of Silver nanoparticles (AgNPs), and (B) XRD of Copper oxide nanoparticles (CuO- NPs).

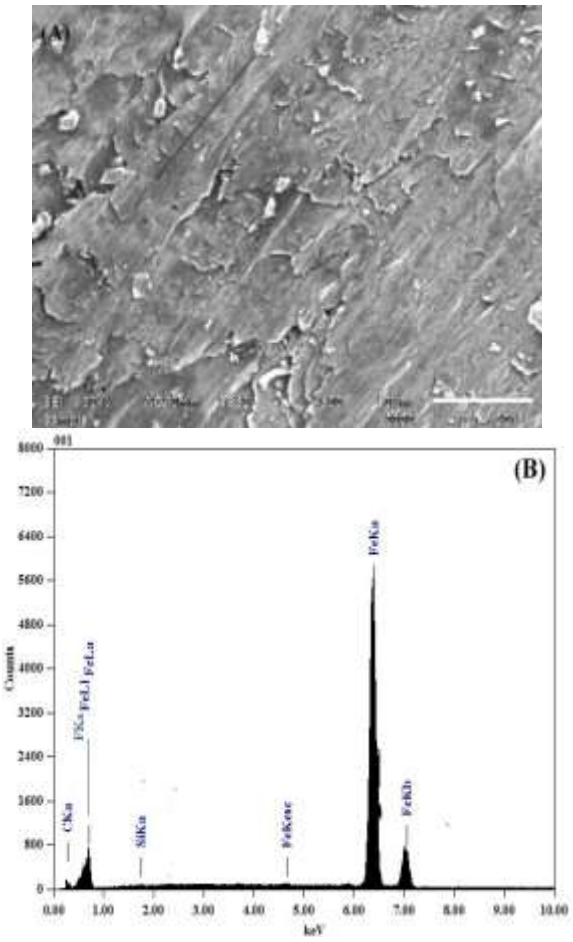

Figure 6: (A) SEM image of the wear formed on the blank pin (metal to metal), (B) EDX spectrum performed on wear on the blank (metal to metal).

L_2) and Ag-NPs (L_1); however, the Cu-NPs were the lowest. This means that the use of Cu-NPs as nano- additives is the best choice to reduce both friction and the wear behavior.

These results were confirmed by the morphological characteristics and chemical constituents of the worn surfaces on the steel pin using SEM and EDX (Figures 4 to 8). These figures showed that there are many deep pits and large area exfoliation on the worn surface in the case of blank indicating that severe adhesive wear had occurred, while the pin surfaces lubricated in cases of L, L_1, L_2 and L_3 were smooth. In particular in the case of L_2 (grease with Cu-NPs), the pin surface was the smoothest as compared with L, L_1 and L_3.

Table 4 showed the chemical composition of the worn surface of pin by EDX. The lubricating grease with Cu-NPs (L_2) had a marked improvement in tribological properties as compared with L, L_1 and L_3. The Cu content in tribochemical film constitutes was 0.23% mass, while Al and Ag were 0.10 and 0.22% mass, respectively. This confirmed that Cu-NPs can adhere to and significantly protect the steel pin compared to other NPs. Also, in the case of L_2 and L_3, the appearance of oxygen element confirms the presence of these nanoparticles in the oxide form. These results are in line with the result of Wang et al. (2007) who reported that the tribological properties of the prepared CaF2 nanocrystals as an additive in lithium grease exhibit excellent anti-wear, friction reduction and extreme pressure (EP) properties (Wang et al., 2007).

Photodegradation of p-nitrophenol (PNP) using nanoparticles

In this study, the results showed a low degradation percentage of P-nitrophenol in the absence of irradiation. The degradation was 4.5%, where PNP was adsorbed on the surface of the Ag-Cu/Al photocatalyst, while it was only 2.3% in the case of using Cu/Al photocatalyst. The percentage of photodegradation of PNP was calculated using the equation:

Photodegradation (%) = (C0-C)/C0 × 100%

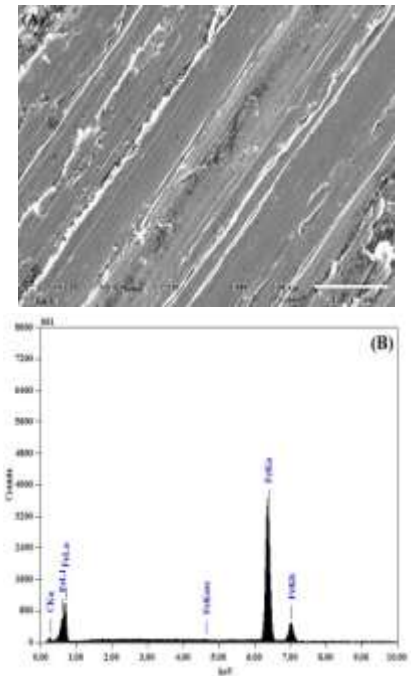

Figure 7: (A) SEM image of the wear formed on the pin lubricated with lubricating grease (L), (B) EDX spectrum performed on wear on the pin lubricated with lubricating grease (L).

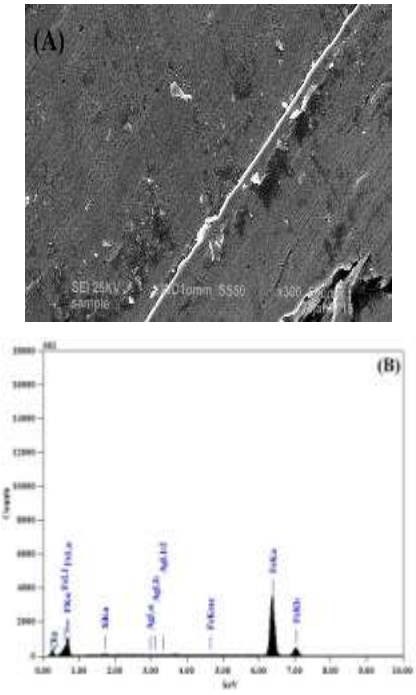

Figure 8: (A) SEM image of the wear formed on the pin lubricated with grease+ AgNPs (L1), (B) EDX spectrum performed on wear on the pin lubricated with grease+ AgNPs (L1).

Table 4: EDX elemental analysis of worn surface of steel pin.

Mass (%)	Blank	Control (L)	L1	L2	L3
Fe K	98.1	97.43	96.64	97.48	96.51
F K	1.48	1.85	2.06	1.61	2.65
Si K	0.19	0.23	0.62	0.31	0.30
O K	0	0	0	0.05	0.09
C K	0.23	0.49	0.46	0.32	0.35
Ag K	0	0	0.22	0	0
Cu K	0	0	0	0.23	0
Al K	0	0	0	0	0.10

Where: L= Pin with lubricating grease only, L1= Pin with lubricating grease + AgNPs, L2= Pin with lubricating grease + Cu-NPs, L3= Pin with lubricating grease + AlNPs.

Table 5: The photodegradation (%) of PNP in case of using Ag-Cu/Al photocatalyst.

Time (h)	Conc. of PNP (ppm)	Photodegradation (%)	C/Co
0	38.2	4.5	0.96
0.5	31.3	21.8	0.78
2	22.6	34.5	0.57
6	17.8	55.5	0.45
10	13.1	67.3	0.33
12	11.7	70.8	0.29

Where: C= Concentration of PNP at time (t), Co= Initial concentration of PNP (40 ppm).

Table 6: The photodegradation (%) of PNP in case of using Cu/Al photocatalyst.

Time (h)	Conc. of PNP (ppm)	Photodegradation (%)	C/Co
0	39.1	2.3	0.98
0.5	32.6	18.5	0.82
2	28.3	29.3	0.71
6	23.7	40.8	0.59
10	20.4	49.0	0.51
12	18.3	54.3	0.46

Where: C=Concentration of PNP at time (t), Co=Initial concentration of PNP (40 ppm).

Where: C0 was the initial concentration of PNP and C, the concentration of PNP at time (t).

The results in Tables 5 and 6 showed the degradation efficiency of PNP after illumination for 12 h. The photocatalytic degradation on Ag-Cu/Al catalyst steadily increased from 54.3 to 70.8% in when compared with Cu/Al catalyst after 12 h illumination. This result was attributed to the presence of AgNPs which acts as an efficient separation center of the photo generated electrons from conduction band and holes from valence band of CuO (Abdul et al., 2016).

Conclusion

The development of a reliable and eco-friendly process for the synthesis of metallic nanoparticles is critically needed in the field of nanotechnology. The current study showed that fungi can produce nanoparticles through green methodology, thus, avoiding the presence of hazardous, toxic solvents and wastes. The method presented a simple process for the myco-synthesis of Ag, Cu and Al nanoparticles with small average size and high monodispersity through reduction of toxic metal salts and *A. niger*, respectively using enzymes and active metabolites of *A. alternata*, *P. duclauxii*.

The biosynthesized NPs were structurally characterized by UV, TEM, DLS and XRD. The optimum conditions for high production of NPs were found to be agitation speed of 150 rpm, a temperature of 28°C, a substrate concentration of 10 mM, fungal gross weight of 10 g and a pH of 6.0. The obtained results suggested that myco-synthesized

nanoparticles can offer a promising eco-friendly and an alternative way to the chemical and synthetic methods. Finally, these NPs could have wide applications in different fields, including enhancement of the tribological properties of machines and as photo-catalysts.

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