MERIT RESEARCH JOURNALS
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Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 4(1) pp. xxx-xxx, January, 2016
Available online http://www.meritresearchjournals.org/mms/index.htm


*Original Research Article*

# Synergistic Effect of Silver Nanoparticles and Laser Light: A Novel Therapeutic Approach on *Aspergillus Fumigatus*

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**Abstract**

**The application of nanoscale materials, ranging from 1 to 100 nm, is an emerging area of nanoscience and nanotechnology. Silver nanoparticles are used in many consumer applications, mostly because of its well documented and safe uses as an antimicrobial agent. This article explores the effect of silver nanoparticle prepared chemically and biologically – enhanced laser therapy against pathogenic *Aspergillus fumigatus*. Previously, physiochemical (X-ray diffraction, ultraviolet-visible absorption, FTIR, and fluorescence spectroscopy) and Transmission Electron Microscopy (TEM) techniques were used to characterize silver nanoparticles were studied. Four different diode lasers with different wavelengths (445, 532, 660 and 808 nm) were used. At fluence rate of 300 mW/cm$^2$ was adjusted for all wavelengths at 10 min of irradiation. 300 mW/cm$^2$ had fluences at 10 min with energy equal 180 J/cm$^2$. Fungicidal and fungistatic effects of various laser light and nano extracts on the growth of *A. fumigatus* were reported; also the effects of fungal morphological features were examined using Image analyser. The results declared that 445 nm was the best laser wavelength, had fungicidal and/or fungistatic potency followed by 660 nm as well as Ag-NPs extract from *P. variotii* was the best Ag-NPs used to prevent the growth of *A. fumigatus* with 445 and 660 nm. More studies on this field were required and application *in vivo* is recommended.**

**Keywords:** *Aspergillus fumigatus*, laser therapy, nano particles, nanotechnology

## INTRODUCTION

Fungi are eukaryotic organisms and their similarities to mammalian cells have led to significant difficulties in the development of new antifungal drugs. Fungal infections are an important health problem worldwide, affecting both immune-competent and immune-compromised individuals. Acquisition of fungal pathogens results in varied outcomes ranging from asymptomatic infection to rapidly lethal systemic disease (Cowen, 2008). Though cutaneous mycoses are rarely life-threatening; they result in significant morbidity, causing discomfort, disfigurement, social isolation, and may predispose to bacterial diseases (Brown *et al*., 2012). These mycoses are frequently recurrent and chronic. Moreover, they are extremely common as it is estimated that 10–20% of the worldwide population may be affected (Drake *et al*., 1996; El-Gohary *et al*., 2014).

In particular, because of the recent advances in research on metal nanoparticles, Ag-Nps have received special attention as a possible antimicrobial agent (Lee *et al*., 2008). It has been known that silver and its compounds have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities for bacteria, fungi, and virus since ancient times (Lok *et al*., 2006).

Compared with other metals, silver exhibits higher toxicity to microorganisms while it exhibits lower toxicity

to mammalian cells (Zhao and Stevens, 1998).

Lately, the recent advances in researches on metal nanoparticles appear to revive the use of Ag-Nps for antimicrobial applications. It has been shown that Ag-Nps prepared with a variety of synthetic methods have effective antimicrobial activity (Kim *et al*., 2008). Hence, Ag-Nps have been applied to a wide range of healthcare products, such as burn dressings, scaffold, water purification systems, and medical devices (Thomas *et al*., 2007). The toxic effects of silver on bacteria have been investigated for more than 60 years. And the acting mechanism of silver has been known in some extent (Rai *et al*., 2009). Therefore, the preparation of uniform nano- sized silver particles with specific requirements in terms of size, shape, and physical and chemical properties is of great interest in the formulation of new pharmaceutical products (Brigger *et al*., 2002).

*Aspergillus fumigatus* is a saprotroph fungus, and is one of the most common species found in soil and dead organic materials, such as compost heaps. In 1992, invasive aspergillosis was responsible for approximately 30% of fungal infections in immunosuppressed patients dying of cancer. It is estimated that invasive aspergillosis occurs in 10 to 25% of all leukemia patients, resulting in a mortality rate of 80 to 90%. These fungi are easily eliminated by the immune system of healthy individuals. However, it causes asthma, allergic sinusitis, and alveolitis in both healthy and unhealthy individuals. *A. fumigatus* is a thermo-tolerant fungus and can grow at maximum temperatures up to 70°C (or 158°F). Due to its wide temperature range for growth, it is not limited to habitats with permanently high temperatures, even though this is very frequently reported in indoor air (Hohl and Feldmesser, 2007).

The majority of work with laser light has been directed at neoplasms, with much less known about the effect of laser on fungi. The majority of work that has been done on fungi has been performed on non-virulent organisms (John, 2010). Localized *Aspergillus fumigatus* infection in the lung, sinuses and brain are difficult to treat, frequently fatal and increasing in frequency. Demand for new treatments for this pathogenic organism is underscored by the emergence of drug resistant strains.

The present paper reviews the research published to date on the effect of different wavelengths of diode laser using silver nanoparticles prepared by biologically and chemically methods on *Aspergillus fumigatus* in an *in vitro* system.

## MATERIALS AND METHODS

### Organisms

*Aspergillus fumigatus* species used in this study was previously isolated from a patient suffering from inflammation of the respiratory disease. Identification of fungal isolate occurred in the Regional Center for Mycology and Biotechnology (RCMB) at Al- Azhar University.

### Inoculum preparation

Fungal isolate was sub-cultured from vial stock onto Sabouraud dextrose agar (Difco, Detroit, USA) and incubated for 48h at 37°C.

### Irradiation source

A GaAlAs diode laser (Photon Laser III, DMC, São Carlos, Brazil) with four wavelengths 445, 532, 660 and 880 nm were used in this study. Fungal isolate was continually irradiated from the top of a flat bottomed micro-titer plaque and the laser beam passed through all the suspensions at 1.0 $cm^2$ spot size, which was coincident for all groups. Fluence rate of 300 $mW/cm^2$ was adjusted for all wavelengths at 10 min of irradiation. 300 $mW/cm^2$ had fluences at 10 min equal 180 $J/cm^2$.

### Nano extracts

Silver nano particles (Ag-Nps) used in this study prepared by different two methods A: chemically and B: myco-biologically.

### A-Synthesis of Silver Nanoparticle (Ag-Nps$^C$) by chemical method

The synthesis of Ag-citrate was done according to the literature procedure Pradeep and Anshup (2009**)**. The synthesis involves the following materials and methods: Silver nitrate $AgNO_3$ and trisodium citrate $C_6H_5O_7Na_3$ of analytical grade purity were used as starting materials without further purification. The silver colloid was prepared by using chemical reduction method. All solutions of reacting materials were prepared in distilled water.

1- 50 ml of 0.001 M $AgNO^3$ was heated to boiling.

2- To this solution 5 ml of 1 % trisodium citrate was added drop by drop. During the process solution was mixed vigorously.

3- Solution was heated until color's change is evident (pale yellow).

4- Then it was removed from the heating element and stirred until cooled to room temperature.

5- Mechanism of reaction could be expressed as follows:

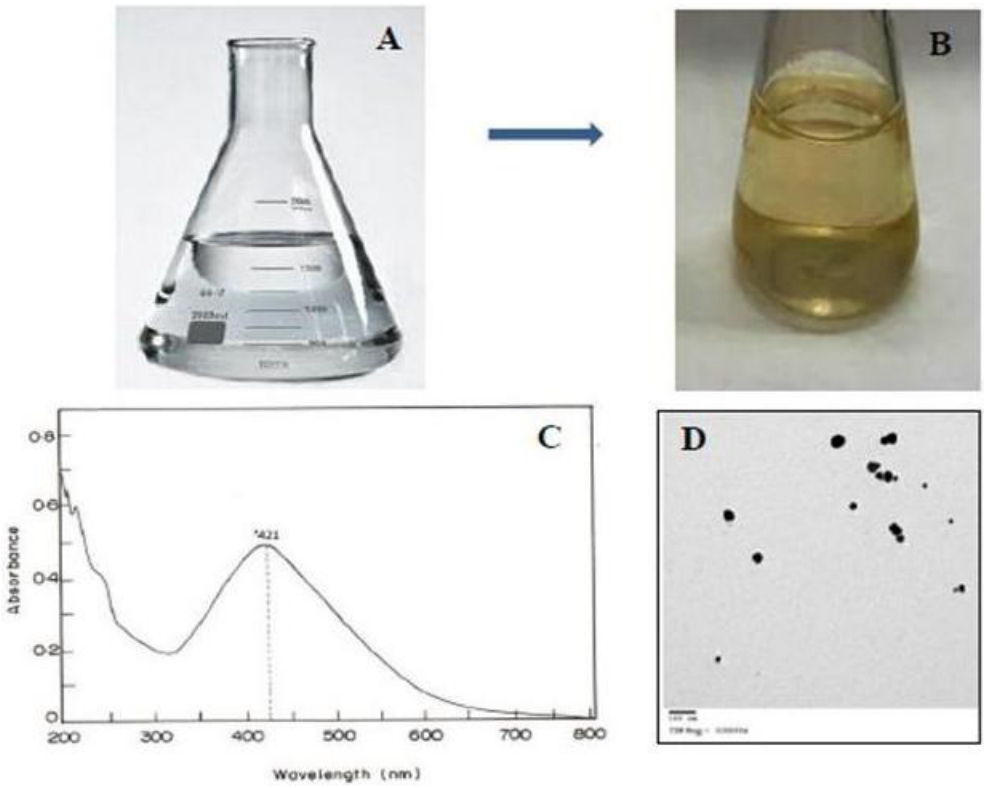


**Figure 1.** Color of solution (A): before and (B): after nano particles formed; (C): UV-Vis spectrum of silver Nanoparticles at 420 Nm; (D): TEM images of silver nano particles.

$$4Ag^{+} + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2\uparrow$$

**B-Synthesis of Silver Nanoparticle by Myco-Biological Method**

Two fungal species (*Aspergillus niger* NRRL 595, and *Paecilomyces variotii* RCMB 200100) were previously subjected for the extracellular biosynthesis of Ag- Nps and characterized using UV– Visible Spectral Analysis, FT-IR, EDX, XRD and MET analyses in an earlier work (Helmy and Mekawey, 2014).

To prepare the biomass for biosynthesis of silver nanoparticles, the fungi obtained were grown aerobically in liquid malt extract medium in flasks at 28±2°C, with shaking using orbital shaker at 150 rpm. The biomass was harvested after 5 days of growth by filtration, followed by extensive washing with sterile deionized water to remove any medium components from the biomass. The broth medium containing the fungal secondary metabolites was used as a control. While, the biomass was divided into two parts: the first one (~10 g) of biomass (wet weight) was brought into contact with 100 mL sterile deionized water and mixed with a carefully weighed quantity of silver nitrate ($AgNO_3$) to yield an overall Ag+ ion concentration of 10 mM (solution A), and the reaction was carried out under dark conditions (Singh and Raja, 2011 and El-Batal *et al*., 2013).

The other part of the biomass (~10 g) was put in 100 ml deionized water without $AgNO_3$ (solution B). After incubation for 72 hours at 37±2 °C and agitated 150 rpm, the solutions (A and B) were filtered and the pH was adjusted to 7.2. Solution B was then divided into two parts: one of them leaves as it is, while the other mixed with $AgNO_3$ and incubated for 10 hours (solution C). Changing of filtrate color from pale yellow to dark brown confirms the Ag-Nps formation (figure 1 "A and B").

Synthesized Ag-Nps from two ways biologically and chemically were confirmed by sampling the brownish aqueous component and the absorption maxima were scanned by UV- visible spectrophotometer at the wavelength of 420 Nm according to Ammar *et al*. (2013) (figure 1 "C"). Morphology and size determination of the Ag-Nps of solution (C), were investigated by TEM (JEOL JEM 1010) and SEM (JEOL JSM 5500) at RCMB (John, 2010). TEM images of silver nanoparticles exhibited nanoparticles with diameters of (25-30 Nm) with magnification power 10.000xs (figure 1 "D").

**Experimental condition**

The experimental method for the study of laser and Ag-Nps effect is the same method of Joseph *et al*.,

(2001) with a simple modification. Four laser lamps at different wave length (440, 532, 660 and 808 nm) and three nano solution (Ag-Nps$^{C}$; Ag-Nps$^{A}$; Ag-Nps$^{P}$ whereas prepared by chemically; using *A. niger* and using *P. variotii*, respectively) used to study their fungicidal and/or fungistatic effect on growth of *A. fumigatus*.

Four groups were tested in each experiment: (i) no light$^{-}$ / no Ag-Nps$^{-}$; (ii) light* / no Ag-Nps$^{-}$; (iii) no light- / Ag-Nps*; (iv) light* / Ag-Nps* (where; - is not present while * is present).

The density of fungal cultures was measured spectrophotometrically and adjusted to 80% transmittance at 532 nm. A stock culture of 3.5X $10^{5}$ cfu/mL was prepared for each experimental condition. Serial dilutions were performed to give cultures with initial inoculum concentrations ranging from 3.5X$10^{1}$ to 3.5X$10^{5}$cfu/mL. 1 ml from each dilution was taken with 100 µL of each nano extract and subject to different laser light doses for 10 min. Control samples from each dilution were grown to assure inoculation and organism viability. Experimental tubes were incubated under growth conditions for 3h before the initial light treatment. Upon completion of final treatments, all tubes were returned to culture conditions for 72 h, after which time they were scored for ‘‘growth, versus ‘‘no growth, by visual inspection. By 72 h all tubes demonstrated either dense confluent growth or remained completely clear. Tubes that showed no-growth after 72 h were further tested as described below.

**Fungicidal versus fungistatic activity of different laser light and nano extracts**

To determine whether the treatments were fungicidal or fungistatic, treated tubes were used to inoculate fresh cultures. A liquots of 100 µL from the no growth tubes that received the light doses were used to seed three separate cultures containing 10 mL of fresh medium. These were then incubated for 4 days at which time no visible growth in any of the cultures was considered evidence of fungicidal effect in the original no growth tube while visible fungal growth in any of cultures was evidence of fungistatic effect and that examined under image analyzer in RCMB Al- azhar University to study the effect of laser light and nano in the morphological features of *A. fumigatus* (Lee *et al*., 2003).

**Image analyzer system**

image analyzer microscope was used for direct light microscopic examination. Soft imaging system GmbH software (analySIS ® pro ver. 3.0 model 1999 at The Regional Center for Mycology and Biotech-nology AL- Azhar University examined the alteration of morphological features of test fungal isolate. magnification power (X 400) was used after, using either phase-contrast or bright field optics under video camera. 3-5 plates were prepared from each isolate and a minimum of 20 microscopic fields were examined. Direct observations of cultures, with particular emphasis on diameter of each conidiophores, vesicle, sterigmata and conidiospore were showed, methylene blue stain were used (Mekawey, 2005).

**RESULTS AND DISCUSSION**

The results of the treatment using different wavelengths of diode laser light and nano extracts (Ag-Nps), *in vitro* are shown in table (1). All controls showed confluent growth at 72h timepoint. All experimental tubes (light*/Ag-Nps*) that contained nano extracts showed both a light dose and inoculum dependent response. There was moderate killing 3.5X$10^{5}$cfu/mL at a light 445 nm with Ag-Nps$^{P}$. On the other hand, 445 nm and 660 nm wave lengths with Ag-Nps$^{P}$ up to complet killing of 3.5X$10^{1}$ cfu/mL (fungicidal effect which the fungal isolate can not grow after 72hrs incubation time) and that indicating these laser wavelengths are the best wavelengths act as fungicidal agents but 445 nm was the much better wavelength.

Subsequent culture of the no-growth tubes established that the successful treatments were fungicidal rather than fungistatic. All cultures treated with nano extract without laser light and on contrast all cultures treated with laser light without nano extracts did not exhibited great affect on their growth, while great reduction on fungal isolate tested were observed under laser light and nano extracts used indicating that nano extract is necessary for fungicidal effect of enhanced the effect of laser light in the present study.

The effect of laser and Ag-Nps treatments on the morphological feature of *A. fumigatus in vitro* was studied. Tested groups [ (i) no light- / no Ag-Nps-; (ii) light* / no Ag-Nps-; (iii) no light- / Ag-Nps*; (iv) light* / Ag-Nps* (- is not present; * is present)] using the dilution 3.5X $10^{1}$ were illustrated in figures (2,3,4,5 and 6).

Figure 2 (A) showed the normal shape of *A. fumigatus* without any effect (no light- /no Ag-Nps$^{-}$), while Figure 2 (B and C) reported the effect of Ag-Nps$^{A}$ on *A. fumigatus*under light microscope. Many variation on fungus shape such as, sterigmata became swollen by 1.8 µm while vesicle became smaller than normal shape by 0.65 µm. No changes were considered on the shape of *A. fumigatus* when treated with Ag-Nps$^{C}$ except vesicle became smaller by 0.45 µm in diameter (Figure 2 "D") while the vesicle enlarged (by 2.1 µm) and sterigmata became shorter and thinner (0.9X 0.43 µm ) when treated with Ag-Nps$^{P}$ (Figure 2 "E"), in addition, conidia exhibited more enlarged (1.6 µm) than normal shape (Figure 2 "F").

445 nm wavelength of laser light exhibited little effect

**Table 1.** Effect of *in vitro* laser light and Ag-Nps on the growth of *A. fumigatus* after 72 h incubation at 37±2 °C

| Treatments | | Inoculum concentration (cfu/mL) | Serial Dilutions | | | | |
|---|---|---|---|---|---|---|---|
| | | | $3.5X10^1$ | $3.5X14^2$ | $3.5X10^3$ | $3.5X10^4$ | $3.5X10^5$ |
| Laser lights wave lengths | 445 | 445*/Ag-Nps - | ++ | ++ | + | 0 | 0 |
| | | 445*/Ag-Nps$^{C*}$ | ++ | + | 0 | 0 | 0 |
| | | 445*/Ag-Nps$^{A*}$ | +++ | ++ | + | + | 0 |
| | | 445*/Ag-Nps$^{P*}$ | ++++ | +++ | ++ | ++ | ++ |
| | 532 | 532*/Ag-Nps - | +++ | +++ | ++ | + | 0 |
| | | 532*/Ag-Nps$^{C*}$ | +++ | ++ | + | 0 | 0 |
| | | 532*/Ag-Nps$^{A*}$ | ++ | + | 0 | 0 | 0 |
| | | 532*/Ag-Nps$^{P*}$ | +++ | ++ | ++ | + | 0 |
| | 660 | 660*/Ag-Nps - | ++ | + | + | 0 | 0 |
| | | 660*/Ag-Nps$^{C*}$ | ++ | + | 0 | 0 | 0 |
| | | 660*/Ag-Nps$^{A*}$ | ++ | + | + | 0 | 0 |
| | | 660*/Ag-Nps$^{P*}$ | ++++ | +++ | ++ | + | 0 |
| | 808 | 808*/Ag-Nps - | +++ | +++ | ++ | + | 0 |
| | | 808*/Ag-Nps$^{C*}$ | +++ | ++ | + | 0 | 0 |
| | | 808*/Ag-Nps$^{A*}$ | ++ | ++ | + | 0 | 0 |
| | | 808*/Ag-Nps$^{P*}$ | ++ | + | + | 0 | 0 |
| Nano Extracts | Ag-Nps | Laser -/Ag-Nps$^{C*}$ | +++ | +++ | ++ | + | 0 |
| | Ag-Nps | Laser -/Ag-Nps$^{a*}$ | +++ | ++ | ++ | ++ | 0 |
| | Ag-Nps | Laser -/Ag-Nps$^{P*}$ | +++ | +++ | ++ | ++ | + |

(Ag-Nps$^{C}$) : nano particles formed by chemically ; (Ag-Nps $^{A}$): nano particles from *A. niger* ; ( Ag-Nps $^{P}$): nano particles from *P. variotii* ; (-) ; not used : (*) : used ; ++++ no growth in all tubes ; +++ no growth in 75% tubes ; ++ no growth in 50% tubes ; + no growth in 25% tubes ; 0 growth in 75% tubes

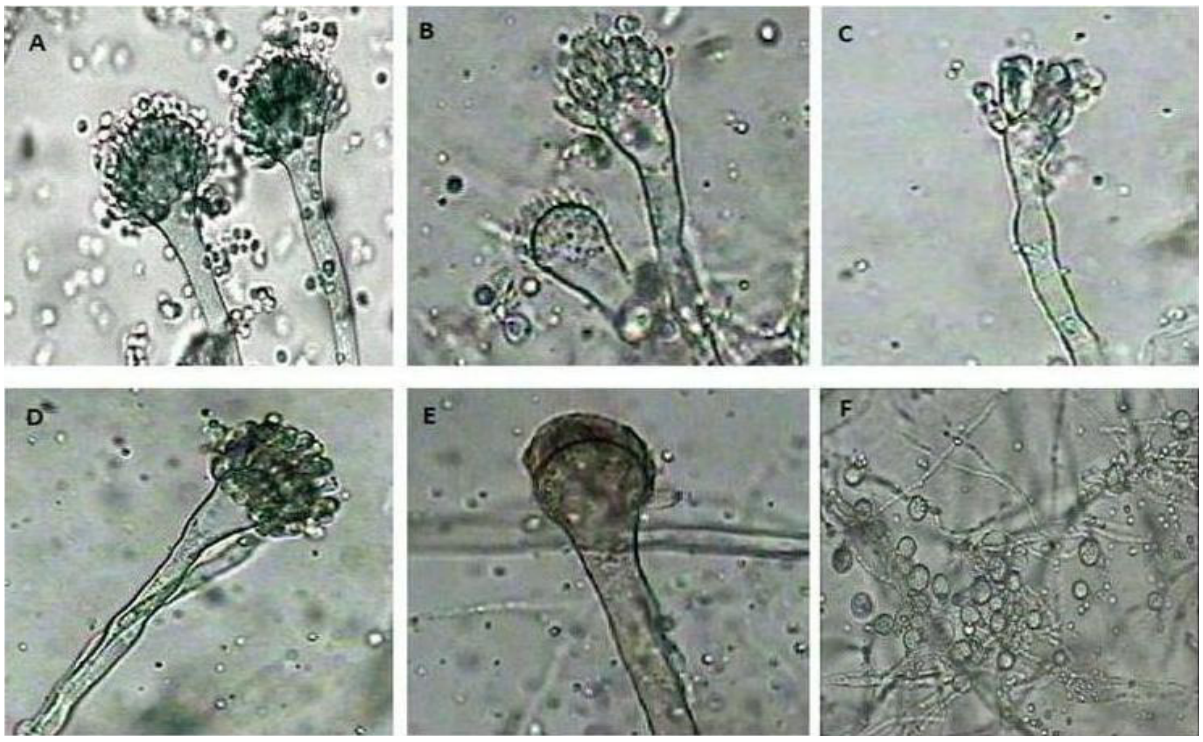


**Figure 2.** *Normal shape of A. fumigatus* under image analysis at (X 40) and with different Ag-Nps treatment extracts

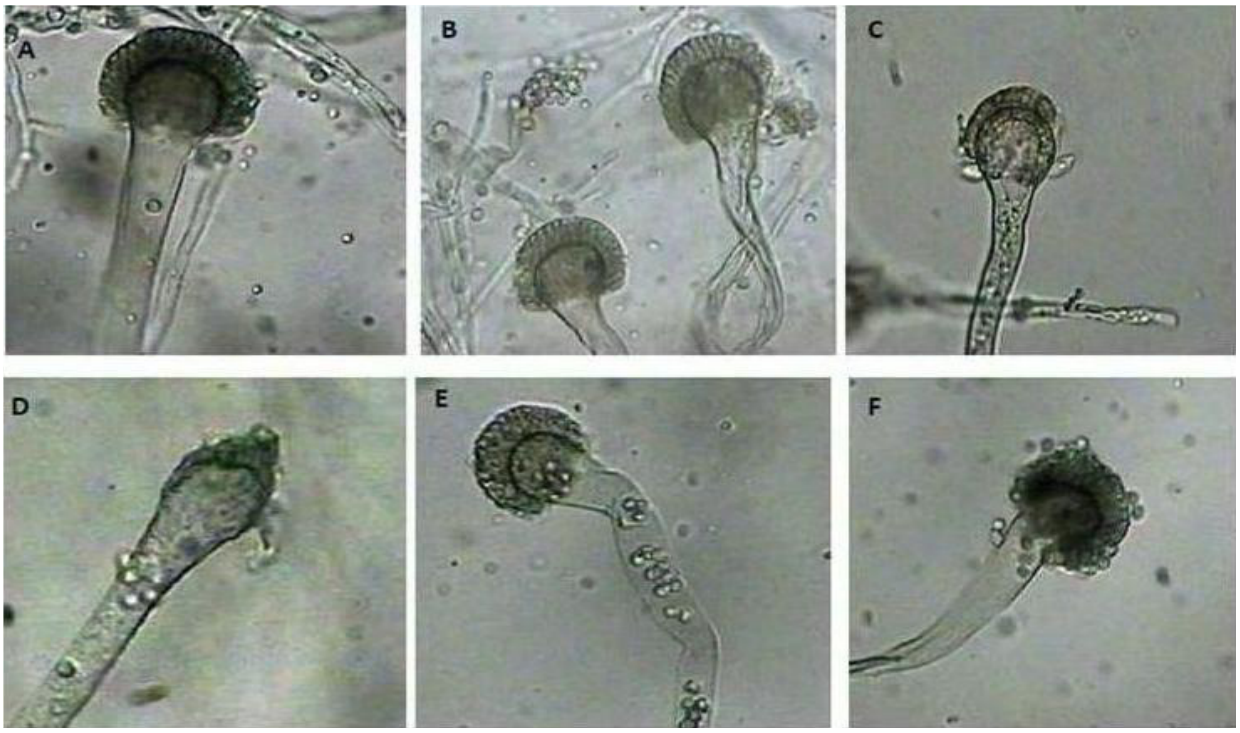


**Figure 3.** *A. fumigatus* under image analysis at (X 40) under 445 nm and different Ag-Nps

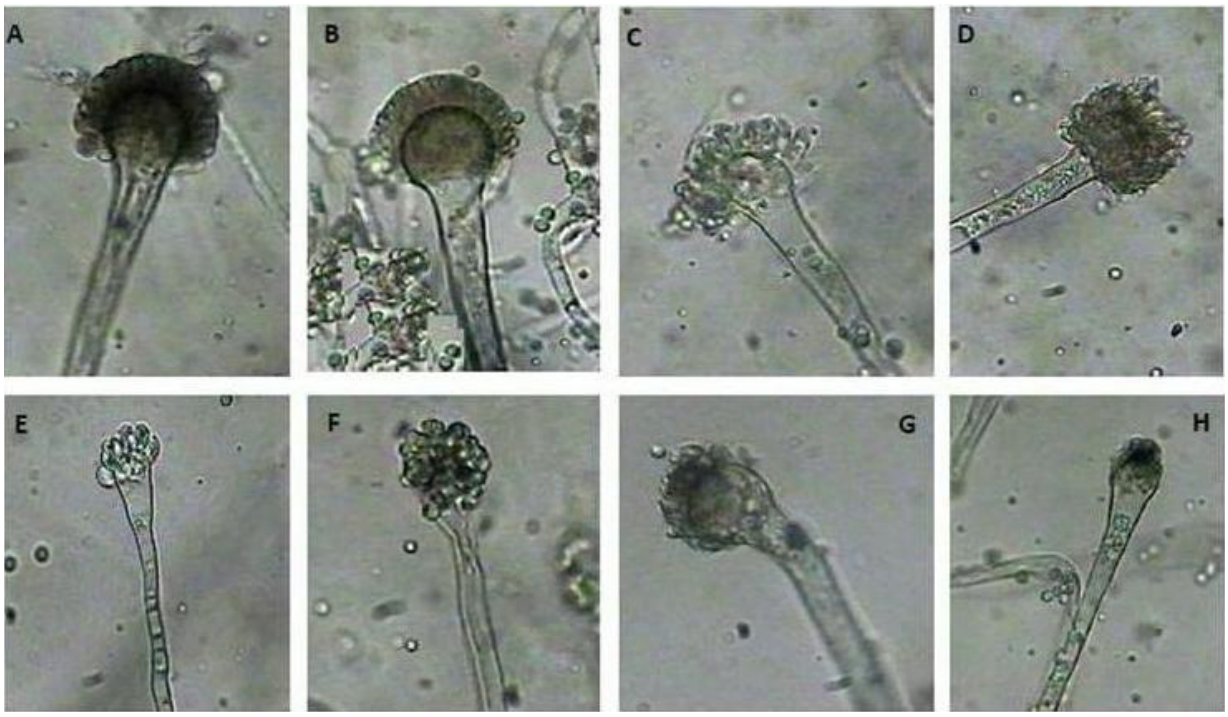


**Figure 4.** *A. fumigatus* under image analysis at (X 40) under 532 nm and different Ag-Nps

on *A. fumigatus* where only vesicle enlarged in size by 2.6 µm (Figure 3 "A and B"), on contrast, great distortion showed after fungus treated with the same laser wavelength and Ag-Nps$^{A}$ which vesicle and sterigmata became much small in size and different in shape (Figure 3 "C and D"). As well as, fungal conidiophore became

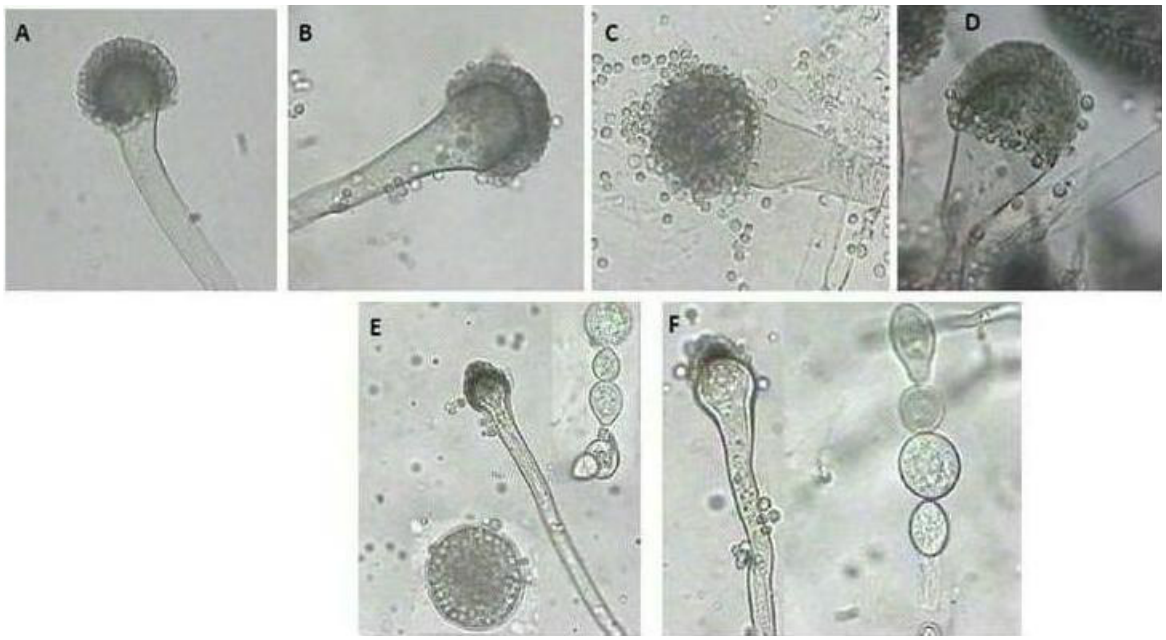


**Figure 5.** *A. fumigatus* under image analysis at (X 40) under 660 nm and different Ag-Nps

swollen in diameter and vesicle was changed in shape after subject to laser wavelength 445 nm and Ag-Nps$^{C}$ (Figure 3 "E and F").

Important to clear that, no fungal growth was obtained after 72h incubation when subject to laser light 445 nm wavelength and Ag-Nps$^{P}$ and that indicated for fungicidal effect of this laser dose and nano particles extracted from paceliomyces fungus on *A. fumigatus*.

As well as, 532 nm wavelength of laser light also exhibited no effect on morphological structure of *A. fumigatus* (Figure 4 "A and B"), while distorsion in vesicle and sterigmata in shape were reported under the same laser wavelength and Ag-Nps$^{A}$ (Figure 4 "C and D").

Great reduction in all fungal organs diameters under 532 nm laser wavelength and Ag-Nps$^{C}$ (Figure 4 "E and F") and with Ag-Nps$^{P}$ (Figure 4 "G and H"), also sterigmata became distorted and more swollen in Ag-Nps$^{C}$ (Figure 4 "E and F").

However, diode 660 nm of laser wavelength did not showed significant effect on the morphological features of *A. fumigatus* under light microscope (Figure 5 "A and B"), while this wavelength showed great distortion on the tested fungus when gave to Ag-Nps$^{A}$ such as, conidiophore and vesicle where enlarged in size by 1.2 and 3.5 µm, respectively and sterigamata became shorter in length by 0.7 µm (Figure 5 "C and D"). As well as, chlamydospores were only observed under this laser wavelength but when gave Ag-Nps$^{C}$ and all fungal organs showed smaller in diameter (Figure 5 "E and F").

Also, important to declare that, the results of this study detected the second diode laser dose exhibited fungicidal effect on *A. fumigatus* with nano extract, whereas, 660 nm wavelength with Ag-Nps$^{P}$ showed complete inhibition of the growth of tested organism after incubate time at 72 h at 37 $^{o}$C and that the same Ag-Nps showed the same fungicidal effect with 445 nm where previously detected.

Finally, laser wavelength at 808 nm did not show any effect on the shape of fungus (Figure 6 "A and B") while all fungal organs, vesicle and sterigmata showed smaller than normal shape when Ag-Nps$^{A}$ used with this laser dose (Figure 6 "C and D"), on contrast, vesicle enlarged in size and sterigmate became thinner and shorter when Ag-Nps$^{C}$ used (Figure 6 "E and F").

Although, Ag-Nps$^{P}$ showed fungicidal effect with 445 nm and 660 nm whereas fungal growth completely inhibited, and exhibited great effect on morphological features of *A. fumigatus* when used with 532 nm laser dose, it did not show any effect on the shape of *A. fumigatus* when used with 808 nm laser wavelength (Figure 6 "G and H").

The findings from this study demonstrate that the laser light can have a significant effect against *A. fumigatus in vitro*. It may have potential as a treatment option for select localized fungal infections that occupy cavities and lend themselves to minimally invasive access, which could be used for instillation of photosenitizer and introduction of a light delivery fibre (Latgé, 1999 and Hohl *et al*., 2007).

Further studies to identfy or develop the optimal Ag-Nps for this application would be required, but the potential use of a non toxic solution that has *in vitro* activity against *A. fumigatus* is observed by Ag-Nps.

Furthermore, the light doses employed for this *in vitro* study are similar to those used clinically for laser and nano particles treatments of endobronchial lung cancer (Latge, 1995 and O'Gorman *et al*., 2008). The

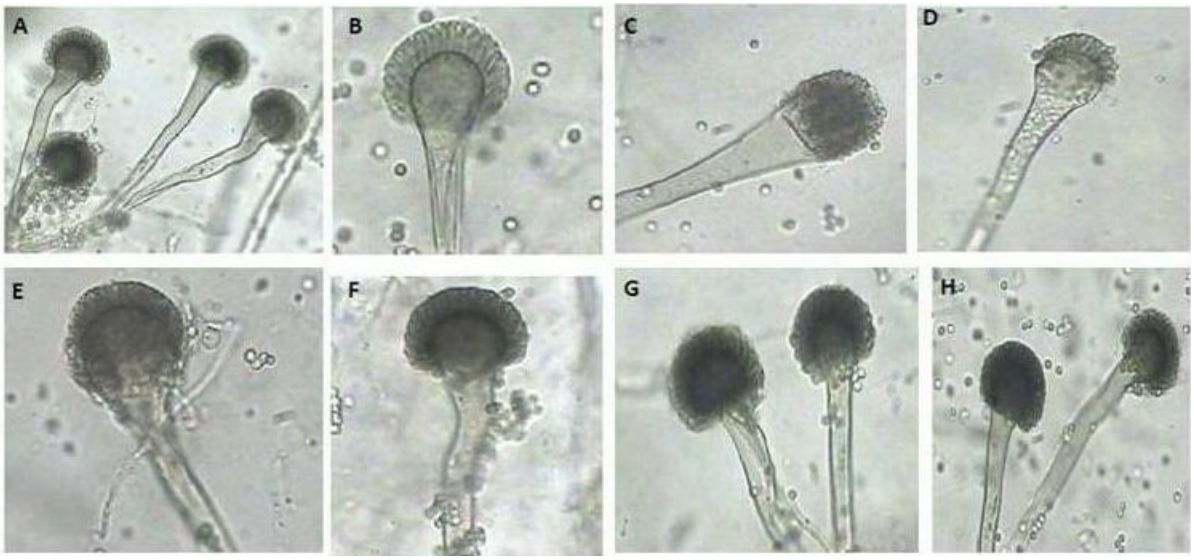


**Figure 6.** *A. fumigatus* under image analysis at (X 40) under 808 nm and different Ag-Nps

demonstration of the fungicidal effect on *A. fumigatus*, in this *in vitro* system, is sufficient to merit further investigation of this antibiotic independent treatment technique.

## CONCLUSION

In conclusion, laser light and nanoparticles from fungi seems to be an alternative to treat dermal aspergillosis, respiratory and nails disease; however, more effective parameters must be found for *in vivo* studies.

### REFERENCES


Ammar A, AA Berhe, TA Ghezzehei (2013). A new method for rapid determination of carbohydrate and total carbon concentrations Using UV spectrophotometry. Carbohydrate Polymers 97: 253–261

Brigger I, C Dubernet, P Couvreur (2002). Nanoparticles in cancer therapy and diagnosis. Adv Drug Deliv Rev, 54: 631–651.

Brown GD, DW, Denning NA, Gow SM, Levitz and T.C. White (2012). Hidden killers: human fungal infections. Sci. Transl.Med. 4, 165.

Cowen LE (2008). The evolution of fungal drug resistance: modulating the trajectory from geno type to phenotype. Nat.Rev.Microbiol. 6, 187–198.

Drake LA, SM Dinehart, ER Farmer, RW Goltz, MK Hordinsky (1996). Guide lines of care for superficial mycotic infections of the skin: tinea capitis and tinea barbae. American Academy of Dermatology. J. Am.Acad.Dermato, 34: 290–294.

El-Batal AI, MA Amin, MK Mona, MA Merehan (2013). Synthesis of silver nanoparticles by*Bacillus stearothermophilus* using gamma radiation and their antimicrobial activity. World Applied Sciences Journal, 22 (1): 01-16.

El-Gohary M, EJ Van Zuuren, Z Fedorowicz, H Burgess, B Stuart (2014). Topical antifungal treatments for tinea cruris and tinea corporis. Cochrane Database Syst.Rev. 8.

Helmy EA, AA Mekawey (2014). Envision of the Microbial Contact with Mycosynthesized Silver Nanoparticles. Res. J. Pharm. Biol. Chem. Sci. 5(5):344-354

Hohl TM, M Feldmesser (2007). *Aspergillus fumigatus:* principles of pathogenesis and host defense. Eukaryotic cell, 6(11): 1953-1963.

John C (2010). Extending the unsharp mask image processing filter. Materials Science and Engineering Dept. USA, pp.1-17.

Joseph SF, C Skama, DB Eric (2001). *In vitro* effect of photodynamic therapy on *A. fumigatus.* J. Antimicronial Chemotherapy, 48:105-107.

Kim JY, C Lee, M Cho, J Yoon (2008). Enhanced inactivation of *E. coli* and MS-2 phage by silver ions combined with UV-A and visible light irradiation. Water Res, 42:356–362.

Latge J (1995). Tools and trends in the detection of *Aspergillus fumigatus.* Current topics in medical mycology, 6: 245.

Latgé JP (1999). *Aspergillus fumigatus* and aspergillosis. Clinical microbiology reviews. 12(2): 310- 350.

Lee BU, SH Yun, J Ji (2008). Inactivation of *S. epidermidis, B.subtilis,* and *E. coli* bacteria bioaerosols deposited on a filter utilizing airborne silver nanoparticles. J. Microbiol. Biotechnol,18: 176-182.

Lee DG, HK Kim, SA Kim, S Jang (2003). Fungicidal effect of indolicidin and its interaction with phospholipids membranes. Biochem Biophys. Res Commun, 305: 305–310.

Lok CN, C Ho, R Chen, QY He, CM Chen (2006). Proteomic analysis of the mode of anti- bacterial action of silver nanoparticles. J. Proteome. Res , 5: 916–924.

Mekawey AAI (2005). Antifungal agents from certain plants: Ph.D of microbiology.. Department of Botany, Girls College for Arts, Science and Education Ain Shams University

O'Gorman CM, HT Fuller, PS Dyer (2008). Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. Nature, 457(7228):471-474.

Pradeep T, S Anshup (2009). Noble metal nanoparticles for water purification: A critical review. Thin Solid Films 517: 6441–6478

Rai M, A Yadav, A Gade (2009). Silver nanoparticles as a new generation of microbials. Biotechnol. Adv, 27:76–83.

Singh P, RB Raja (2011). Biological synthesis and characterization of silver nanoparticles using the fungus *Trichoderma harzianum* Asian J. Exp. Biol. Sci., 2(4): 600-605.

Thomas V, M Yallapu, B Sreedhar, SK Bajpai (2007). A versatile strategy to fabricate hydrogel– silver nano composites and investigation of their antimicrobial activity. J Colloid Interface Sci, 315: 389–395.

Zhao GJ, SE Stevens (1998). Multiple parameters for the comprehensive evaluation of the susceptibility of *Escherichia coli* to the silver ion. Biometals,11: 27–32.