Egypt. Acad. J. Biolog. Sci., 12(1): 29-43 (2020)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
www.eajbs.eg.net

The Efficiency of Biosynthesized Silver Nanoparticles by Endophytic *Fusarium chlamydosporum* F25 against Plants Postharvest Fungal Pathogen

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ARTICLE INFO
Article History
Received: 12/2/2020
Accepted:9/3/2020

***Keywords*:**
Endophytic fungi, *Fusarium chlamydosporum*, nanosilver particles, plant pathogen, postharvest disease

ABSTRACT

Silver nanoparticles (AgNPs) were evaluated for its possible controlling postharvest pathogen. Ten endophytic fungi isolated from medicinal plant (*Calotropis procera* (Ait.) R. Br.), out of these isolates only one can biosynthesizes silver nanoparticles. The isolate was identified as *Fusarium chlamydosporum* F25 according to sequence similarities and phylogenetic analysis. The Silver nanoparticles were characterized by Transmission Electron Microscope (TEM), Scanning Electron Microscopy (SEM) and Energy Dispersive Analysis of X-ray (EDX). Four postharvest pathogenic fungi were isolated and identified according to morphological, and microscopical characteristics**,** the isolated fungi were identified as *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus niger*, and *Penicillium digitatum.* The antifungal activity of silver nanoparticles was tested against the isolated pathogens. *In vitro* silver nanoparticles had a significant effect on growth of all pathogens. Furthermore, silver nanoparticles used to control the postharvest green mould disease of orange caused by *Penicillium digitatum.* Commercial fungicide Revus top used as a positive control. Silver nanoparticles showed high efficiency against the disease. This study provides the possibility of the use of silver nanoparticles as a protectant fungicide against postharvest disease.

INTRODUCTION

Medicinal plants are reported to harbor endophytes (Strobel, 2002), which in turn provide protection to their host from infectious agents and also provide adaptability to survive in adverse environmental conditions. *Calotropis procera* (Ait.) R. Br., commonly known as calotrope, rubber tree, and akando, is a widely used medicinal plant in the Indian Sub-continent (Akinloye e*t al*., 2002; Kumar and Roy, 2007). Different parts of the plant have been reported to possess a number of biological activities such as antimicrobial (Sing *et al*., 2002; Khan *et al*., 2007). Nanotechnology is the technology of materials having a particle size below a hundred nanometers. The properties of materials below a hundred nanometers usually differ from those in the bulk scales. Silver nanoparticles (AgNPs) among all noble metals have been widely used in many pharmaceutical and biological applications because of their unique antimicrobial properties (Egger *et al*., 2009).

During biosynthesis of NPs, reduction of precursor (mainly metal salts ion) by reducing agents (a biomolecule or a biological process) normally results in the accumulation of reduced ions and formation of NPs. Therefore, the condition of ion reduction strongly affects the size and shape of NPs. This is the main key factor to control different properties of NPs. Because of the biotechnology abilities, modification in precursor and reducing agent or their interaction condition provides an almost unlimited toolbox for control of NP characteristics, production rats, and also waste minimization (Sharma *et al*., 2009; Naghdi *et al*., 2015). Fungi are commonly used in the biosynthesis of inorganic NPs in comparison to bacteria because of higher output and their easy handling (Iravani, 2014; Hulkoti and Taranath, 2014). The biosynthesis is possible by direct contact of ions with fungi biomass (Ahmed *et al*., 2002; Vahabi*et al*., 2011) or interaction of metal ion with biomass-free extracts (Shankar *et al*., 2004) such as enzymes and other biomolecules secreted from fungi (Mukherjee *et al*., 2001). Management of fungal diseases of food crops and fruits is economically important. Moreover, a higher effort has been given to develop secure management techniques that pose less risk to humans and livestock, with a focus on the consequences of synthetic fungicides. Kim *et al*. (2012); Shams-Ghahfarokhi *et al*. (2014) demonstrated that AgNPs had low toxicity, a broad spectrum of antimicrobial activity and also very effective against plant phytopathogenic fungi. Citrus fruit crop has a tremendous economic, social and health impact on human all over the world. Citrus fruit belongs to genus Citrus and family Rutaceae, include oranges, lemons, limes and grapefruits and widely used as edible fruits. (Sidana *et al.*, 2013). Egypt annually produces about 3 million tons of Citrus fruits, Citrus fruits are attacked by a number of pathogens from bloom to harvesting stage and subsequently by post-harvest pathogens that affect fruit yield and considerably deteriorate the fruit quality these are *Alternaria* sp., *Botryodiblodia theobromae*, *Fusarium sp*., *Penicillium digitatum* and *Penicillium italicum* (Embaby *et al*., 2013; Ammar and El-Naggar, 2014). The main post-harvest diseases of citrus can be divided into two groups based on their initial infections: (i) diseases from field infection such as *Alternaria* rot, Brown rot, Phomopsis, and Diplodia stem-end rot, and Anthracnose; and (ii) diseases due to post-harvest infection such as *Penicillium* decays and *Fusarium* decays (Ippolito and Nigro, 2009). Green, blue, and whisker decays caused by *Penicillium digitatum* (Pers.: Fr.) Sacc., *Penicillium italicum* Wehmer, and *P. ulaiense* (Hsieh, Su, and Tzean), respectively, are the most important post-harvest diseases attacking citrus fruit worldwide (Youssef *et al.*, 2010; Youssef *et al*., 2012). The above post-harvest diseases are usually controlled by synthetic fungicide applications in packing houses. However, several constraints related to their use forced scientists to develop alternative means to control postharvest diseases. This is true that pesticides like fungicides, insecticides, herbicides, etc. effectively play an important role in controlling plant pathogenic organisms but these pesticides also affect soil texture, soil microorganisms and cause water pollution as well as soil pollution. Margni *et al.* (2002) showed that changes in the soil activity depending on the intensity and spectrum of activity as well as persistence of the parent chemicals or its metabolites. Also, Kjoller and Rosendah (2000); Narender (2011) observed that fungicides restricted the development of mycorrhizal fungi. The objectives of this research were carried out to evaluate the antifungal activity of bio-synthesized silver nanoparticles for controlling fungus post-harvest diseases and evaluate the efficiency of commercial fungicides.

MATERIALS AND METHODS

Isolation and Purification of Endophytic Fungi:

Stems and leaves of *Calotropis procera (Ait.) R. Br.* were randomly collected from healthy and mature naturally grown plants. Samples were processed in the laboratory within a few hours to reduce the chances of contamination. Stems and leaves explants were prepared for isolating endophytic fungi according to Hallman *et al.* (2007).

Biosynthesis of AgNPs:

Fungal isolates were grown in 100ml cultures of malt extract broth medium. The flasks were incubated at 28°C for 5days on a rotary shaker (120 rpm). Fungal biomass was separated by filtration, washed with sterile distilled water to remove the traces of culture media components, re-suspended in 100 ml twice distilled water, incubated at 28°C for 24hours, and then filtered. 10 ml $AgNO_3$ solution (1mM) was added to the filtrate and reaction mixture without $AgNO_3$ was used as control. The ratio of cell filtrate to $AgNO_3$ was kept at 1:9 (v/v), and the reaction mixture was incubated at 28°C for 48hours. The AgNPs were purified by centrifugation at 10,000 rpm for 10 min twice and collected for further characterization (Devi and Joshi, 2012).

Identification of Selected Fungus:

The nanosilver (AgNPs**)** producer fungus was identified on the basis of the sequence similarities and phylogenetic analysis in the Sigma company of scientific service, Cairo – Egypt according to White *et al*., (1990); Altschul *et al*., (1997).

Characterization of AgNPs:

After 24 hours of synthesis, the sample of AgNPs was centrifuged at 10,000 rpm for 30 minutes at room temperature. Repeated rinses were performed to remove impurities. The residue of AgNPs was resuspended in 1ml sterile water. The characterization of AgNPs including Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and Energy Dispersive Analysis of X-ray (EDX) which performed at the Regional Centre for Mycology and Biotechnology (RCMB), Cairo – Egypt.

Transmission Electron Microscopy (TEM):

For TEM analysis, a drop of the cell filtrate was placed on the carbon-coated copper grids and dried by allowing water to evaporate at room temperature. Electron micrographs were obtained using GEOL GEM - 1010 transmission electron microscope at 70 kV (Jain *et al*., 2011).

Energy Dispersive Analysis of X-ray(EDX):

The presence of elemental silver was confirmed through EDX. The EDX microanalysis was carried out by X-ray microanalyzerer (Oxford 6587 INCA) attached to JEOL JSM-5500 LV scanning electron microscope at 20 kV. (Devi *et al*., 2012 and Shoeb *et al*., 2013).

Scanning Electron Microscopy (SEM):

The scanning electron microscopy (SEM) was carried out using a fine powder of the AgNPs on a carbon tape in (JOEL JSM-5500LV). SEM was performed at Regional Center for Mycology and Biotechnology (RCMB) at AL-Azhar University, Cairo - Egypt.

Isolation and Identification of Postharvest Fungal Pathogens:

The infected tissues of pre-harvest tomato, apple, orange, and pomegranate along with adjacent small unaffected tissues were collected and processed for isolating pathogenic fungi. The isolated fungi were identified based on macroscopic and microscopic characteristics using suitable media, slide cultures and the most updated keys for identifications with sporulation according to Barnett and Hunter (1999); Ara *et al*. (2012); Loliam *et al*. (2012); Venkateswarlu *et al*. (2015).

Standard Fungicide:

Commercial fungicide named Revus top (purchase from Syngenta company) was used as a positive control against AgNPs for control postharvest disease. It is a complex of two active substances; Mandipropamid 25% and Difenoconzole 25% (W/V). The recommended dose was 500 µg/ml. It works efficiently under all suitable and inappropriate weather conditions. Its wide

range of prevention and treatment of various fungal diseases.

***In Vitro* Assay for Antifungal Activity of Biosynthesized AgNPs and Fungicide:**

The antifungal effect of biosynthesized AgNPs and Revus top commercial fungicide (at the recommended dose) was examined against the pathogenic fungal isolates using the agar well diffusion method. The experiment was carried out in triplicates and the average zone of inhibition in mm ±SD was calculated. The lowest concentration of AgNPs that prevented microbial growth represented the minimal inhibitory concentration (MIC) was measured according to Wiegand *et al*. (2008).

Control of Green Mould Disease Using Biosynthesized AgNPs and the Fungicide:

Silver nanoparticles were tested as a postharvest fungicide for testing its efficiency to control the incidence of green mould disease caused by *P. digitatum*. Healthy uniform navel oranges were used in this test. The washed, sterilized fruits were inoculated artificially with *P. digitatum* according to Salem *et al*. (2019). The infection treatment was carried out by dip method. The orange fruits were dipped either in different concentrations of biosynthesized nanosilver particles or in different concentrations of Revus top fungicide. The MIC of AgNPs against *P. digitatum* represent 100% concentration and the recommended dose of fungicide represent 100% concentration. The treatment was tested in three replicate, and each replicate contained ten oranges. After drying the treated fruits were stored in plastic bags and inspection for decay was carried out 15 days of storage at 25°C. The efficacy of each treatment was determined according to the equation described by Samoucha and Cohen, (1989) as follow: PCE = 100 (1-x / y), where PCE percentage of control efficacy; x: number of decayed fruits in nanoparticles or fungicide treatment and y: number of decayed fruits in the control treatment.

Preparation of Fungicide Concentrations:

The recommended dose of the commercial product, 500 µg/mL which represent 100% as higher dose) and two lower other concentrations (50% middle and 25% lower dose) were used.

RESULTS AND DISCUSSION

Biosynthesis of Silver Nanoparticles (AgNPs) of Endophytic Fungal Isolates:

Ten endophytic fungal isolates were isolated and purified from *Calotropis procera* (Ait.) R. Br**.** Out of the 10 fungal species screened, only one fungal species was found to reduce silver salt into silver nanoparticles by visual observation of the fungal filtrates. This fungus filtrate exhibited a gradual change to brown color, clearly indicating the formation of AgNPs. The colour of the culture filtrate with silver nitrate solution changed to intense brown after 24 hr of incubation, whereas, the control (without silver nitrate salt) did not exhibit any colour change. The colour changes observed can be attributed to the surface plasmon resonance of deposited AgNPs (Mulvaney, 1996).

Identification of Selected Fungi:

The fungus was identified on the basis of 18S rRNA gene sequencing and phylogenetic analysis (Figs. 1&2 and Table1). The 18S rRNA gene sequencing was compared with a sequence of *Fusarium* sp. through multiple sequence alignment. Experimental analysis of PCR amplification was studied through agarose gel electrophoresis. The multiple sequence alignment, which showed that the isolate was closed to *Fusarium chlamydosporum* F25by 99% identity with query cover 100%.

Fig. 1. Straight and falcate macroconidia of *F. chlamydosporum*F25 with 2-3 septa per conidium**.**

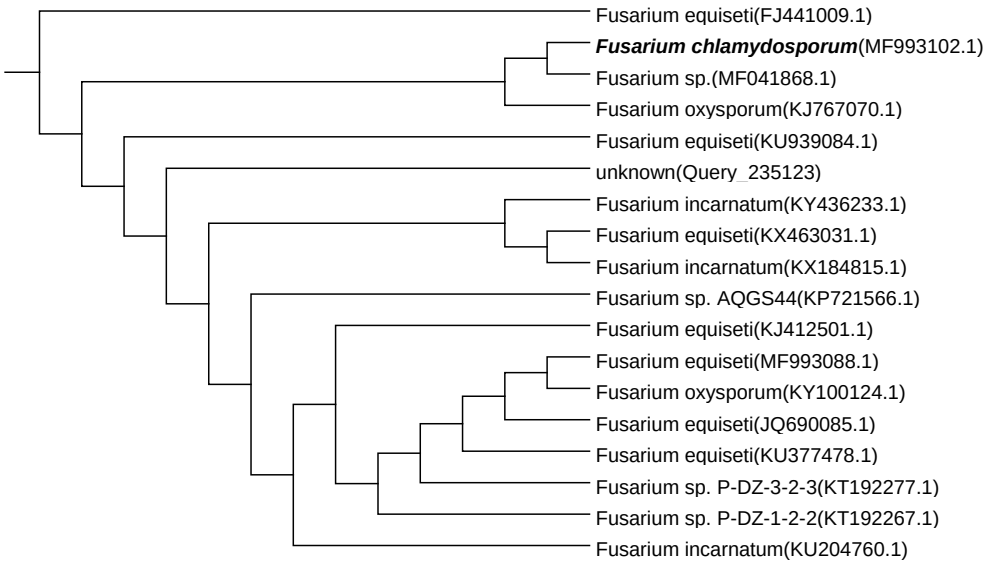

Fig. 2. Neighbor-joining tree based on 18S rRNA gene sequences showing the phylogenetic relationship.

Table 1. Sequence producing significant alignment of *Fusarium chlamydosporum* F25.

Description	Max score	Total score	Query cover	E value	Ident. %	Accession
Fusarium chlamydosporum isolate F25	867	867	100	0.0	99	MF993102.1
Fusarium oxysporum isolate A2s3-D1	867	867	100	0.0	99	KJ767070.1
Fusarium equiseti isolate JG22	867	867	100	0.0	99	KJ412501.1
Fusarium equiseti isolate dx-7 18s	867	867	100	0.0	99	FJ441009.1
Fusarium equiseti isolate F11	865	865	99	0.0	99	MF993088.1
Fusarium equiseti isolate KA	865	865	99	0.0	99	JQ690085.1
Fusarium sp. isolate CRO-IIHR	863	863	100	0.0	99	MF041868.1
Fusarium incamatum strain NBt 1H	863	863	98	0.0	99	KU204760.1
Fusarium sp. AQGS44	863	863	98	0.0	99	KP721566.1
Fusarium incamatum isolate Cjmgnf2	861	861	100	0.0	99	KY436233.1
Fusarium oxysporum strain FSOT	861	861	100	0.0	99	KY100124.1
Fusarium equiseti isolate A577	861	861	100	0.0	99	KX463031.1
Fusarium incamatum isolate HNMi	861	861	100	0.0	99	KX184815.1
Fusarium equiseti isolate 7DF	861	861	100	0.0	99	KU939084.1
Fusarium equiseti isolate strain D014	861	861	100	0.0	99	KU377478.1
Fusarium sp. P-DZ-3-2-3	861	861	100	0.0	99	KT192277.1
Fusarium sp. P-DZ-1-2-2	861	861	100	0.0	99	KT192267.1

Characterization of AgNPs

Microscopic Characterization by TEM

The data obtained from the transmission electron-micrograph showed distinct shape and size of nanoparticles. The particles were spherical in shape, their size was ranging from 7.30 – 11.59 nm**,** with a mean of 9.39 nm (Fig.3a and Table 2). AgNPs uniformly distributed with some agglomeration which revealed a pattern similar to the biosynthesized AgNPs by Kathiresan *et al*. (2009) and Jain *et al*. (2011).

Scanning Electron Microscopy (SEM)

The SEM micrograph shows silver nanoparticles aggregates. In this micrograph observed spherical nanoparticles. The nanoparticles were not in direct contact even within the aggregates (Fig. 3b).

Energy Dispersive Analysis of X-ray (EDX)

EDX gives qualitative, as well as the quantitative status of elements that may be involved in the formation of AgNPs. Figure 3c shows the EDX spectrum recorded in the spot profile mode. The optical absorption peak is observed at 3KeV, which is typical for the absorption of metallic AgNPs by Magudapathy *et al*. (2001). Strong signals from the silver atoms are observed, while weaker signals from cu and Zn atoms are also recorded. From the EDX spectrums, it is clear that AgNPs reduced by *Fusarium chlamydosporum* F25 has the weight percentage of silver as 95.6% (Table3).

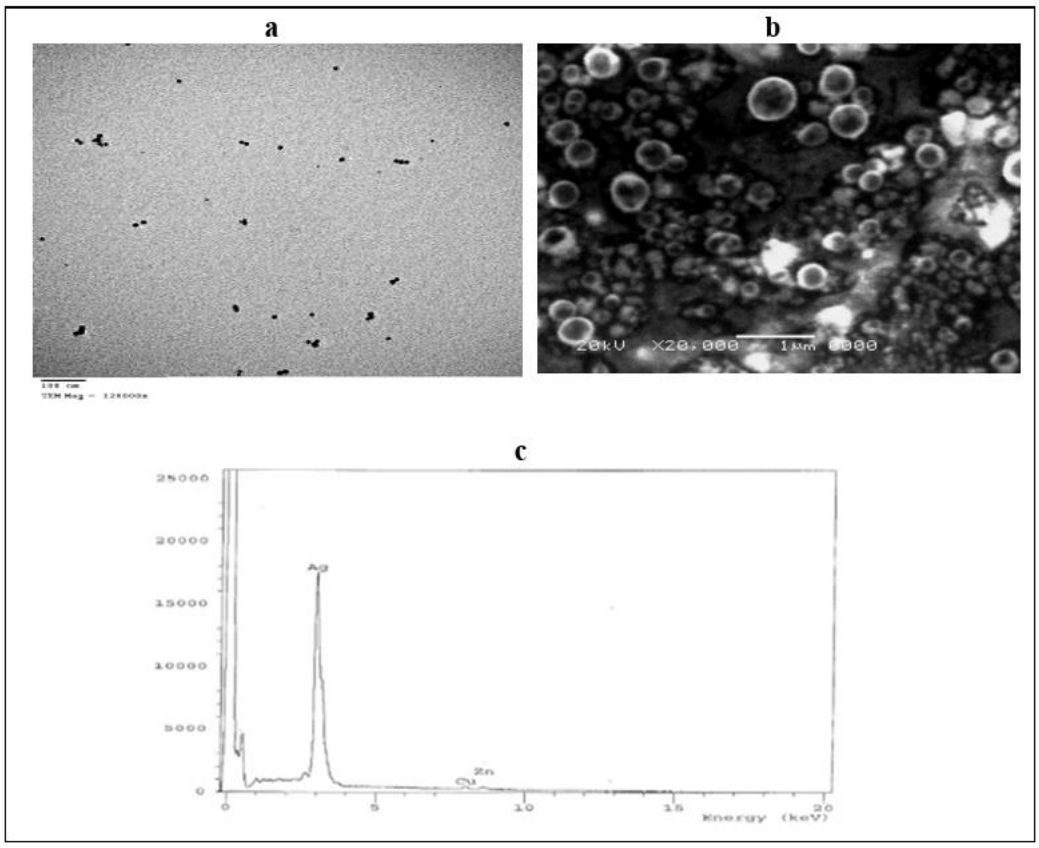

Fig. 3. TEM micrograph (a), Scanning electron micrograph (b), EDX spectra of AgNPs, Silver X-ray emission peaks are labeled (c) of the silver nanoparticles synthesized by *Fusarium chlamydosporum* F25.

Table 2. Statistical measurements of silver nanoparticles (AgNPs)

Statistical function	Distance (nm)
Count	10
Mean	9.39
Maximum	11.59
Minimum	7.30
Standard deviation	1.48

Table 3. The element composition of the AgNPs of EDX spectra

	Element	Min.	Max.	Mean	SD
Element percent (%)	Cu	2.020	2.150	2.080	0.066
	Zn	2.120	2.530	20273	0.224
	Ag	95.40	95.81	95.647	0.217

Isolation and identification of postharvest plant infected fungi

Alternaria, *Fusarium*, *Aspergillus*, and *Penicillium* were isolated from postharvest tomato, apple, orange and Pomegranate fruits, respectively. According to cultural properties and microscopic characteristics**,** the isolated fungi were identified as following (Plate 1);

Alternaria alternata

Colony growth characters; the fungus grows rapidly and colony size reaches a diameter of 3- 8 cm following incubation at 25°C for 7 days on the Potato dextrose medium. The colony is flat, downy to wooly and it is covered by grayish short, aerial hyphae in time the surface is grayish-white at the beginning which later darkens and becomes greenish-black with a light border. The reverse side is typically black due to pigment production. Microscopic examination; *Alternaria* have septate brown hyphae. The average conidial length varied from 15 to 55µm and breadth range from 5.5-14 µm. The transverse and longitudinal septa varied from 2-6 and 0-4, respectively.

Fusarium oxysporum

Colony growth characters; colonies fast-growing, reaching 5-7 cm diameter in seven days at 25°C on PDA media. Aerial mycelium abundantly developed, intensely cotton white accompanying with dark violet pigment on PDA. The average macroconidia length and width varied from 25-50 µm and 3.5-5.2 µm respectively, and have a slightly curved shape with 2-4 septate. chlamydospores formed were relatively abundant in mycelium mostly globose intercalary.

Aspergillus niger

Colony growth characters; Conidia of *Aspergillus niger* are black and densely packed. hyphae inconspicuous, white to dull yellow; exudates were absent; reverse uncolored to florescent yellow and wrinkled mycelial growth. The colony reached 5-7cm diameter in 7 days at 28°C, on Czapek Dox medium. Its microscopic characters revealed radiating conidial heads while the conidiophores will appear, unbranched and uncolored. Sterigmata arranged in a single series, conidia were appeared as sub-globose and round. The conidiophore 9.5 µm in diameter. A vesicle is globose and 28.0 µm. First Sterigmata Uniseriate, 7.7 X 4.5µm and globose sub conidia 3.5 µm.

Penicillium digitatum

Colonies are plane and grow rapidly reached 5-7cm diameter in 7 days at 28°C on PDA. The colony observes is olive green and the reverse colorless to cream yellow. Colony texture is velvety to deeply floccose with no exudate droplets. Conidiophores are irregular branched and branches that terminate in whorls phialides. Conidiophore size range from 65-120 µm in length. Phialides can range in shape from flask-shaped to cylindrical and can be 8–15 µm long. Conidia are variable in size 3-5 µm.

***In vitro* the Antifungal Activity Of Fungicide And Biosynthesized AgNPs and Minimum Inhibitory Concentration (MIC):**

The antifungal activity of mycosynthesized AgNPs at the concentration (100 µg/ml) and tested fungicide were checked against isolated plant pathogens (*Alternaria alternata*, *Fusarium oxysporum*, *Penicillium digitatum*, and *Aspergillus niger*). The results in Table (4) indicated that the biosynthesized AgNPs exhibited promising antifungal activity more than fungicide against all tested fungal isolates, in accordance with several previous reports (Kim *et al*., 2008; Gajbhiye *et al*., 2009; Xue *et al*., 2016; Elamawil *et al*., 2018, Al-zubaidi *et al*., 2019). MICs of silver nanoparticles against pathogenic fungal isolates were studied. The results suggest that the MICs of AgNPs were 3.12-50 µg/ml. The highest MIC was recorded with *P. digitatum* while *F. oxysporum* the most sensitive isolate. Similarly, a recent publication also showed that biosynthesized silver nanoparticles had antimicrobial effects against eighteen plant pathogenic fungi-isolated from spoiled fruits and vegetables (Shams-Ghahfarokhi *et al*., 2014). Several reports on the mechanism of silver ions inhibitory action on microorganisms have shown that DNA loses its ability to replicate upon treatment with Ag^{+} (Feng *et al*., 2000), resulting in inactivated expression of ribosomal subunit proteins, as well as certain other cellular proteins and enzymes essential to ATP production (Yamanaka *et al*., 2005).

Table 4. Antifungal activity of biosynthesized AgNPs and minimum inhibitory concentration (MIC µg/ml) against pathogenic fungi.

Treatment Tested fungi	Inhibition zones (mm) ±SD AgNPs (100 µg/ml)	Revus top fungicide (500 µg/ml)	Minimum Inhibitory Concentration (MIC) of AgNPs µg/ml
Alternaria alternata	27.2±1.2	22.9±0.56	12.5
Fusarium oxysporum	35.56±0.5	30.4±1.1	3.12
Aspergillus niger	28.6±0.5	22.1±0.9	25.8
Penicillium digitatum	27.8±0.8	19.1±0.5	50

*Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6mm) against pathogenic fungal isolates.

Morphological Alteration of Fungi Resulted by AgNPs

During the experimental process, some morphological alterations on fungi were observed (Plate 2). Microscopic analysis was carried out to illustrate this differentiation. Treatment of *Alternaria alternata* with nanoparticle solution induced inhibition of germ tube elongation and mycelial malformation. Nanoparticle solution disrupts the septation of macroconidia of *Fusarium.* Also, the curved shape disappears this may be due to the thickening of macroconidial wall. Vacuolization and hyphae swelling was observed. Regarding *Aspergillus niger* complete inhibition of sporulation was detected. Inhibition of sporulation was observed in *Penicillium*. Durán *et al*. (2005) attributed the antimicrobial activity of AgNPs to the formation of insoluble compounds by inactivation of sulfhydryl groups in the fungal cell wall and disruption of membrane-bound enzymes and lipids resulting in lysis of the cell. It was also reported that the process may involve the binding of AgNPs to external proteins to create pores, interfering with DNA replication or forming reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anions, and hydroxyl radicals (Duran *et al*., 2016; Ottoni *et al*., 2017).

Silver Nanoparticle as A Postharvest Fungicide for Control of Green Mould Caused by *Penicillium digitatum:*

In the present study, the biosynthesized AgNPs show efficiency against four post-harvest plant pathogen. In the present study, *P. digitatum* is the most resistant pathogenic fungi either to AgNPs or fungicide. *P. digitatum* is the causal fungi for green mould postharvest disease of citrus. So, it was chosen for testing the fungicide of nanoparticle efficiency and comparing the results with a commercial fungicide. Revus top fungicide was chosen as a positive control against the biosynthesized nanosilver against the incidence of green mould on orange fruits. It is a complex of two active substances. It works efficiently under all suitable and inappropriate weather conditions. Its wide range of prevention and treatment of various fungal diseases. The orange fruits were dipped either in different concentration of biosynthesized nanosilver particles (100, 50, 25 % MIC) or in different concentration of the fungicide (100, 50 and 25% of recommended dose). Results in Figure (4) showed that the percentage of control efficiency of Ag-NPs reached 75, 84 and 96 % at 25, 50 and 100 % MIC, respectively. On the other hand, the results showed that the fungicide at all concentrations expressed suppressive ability on the disease. The percentage of control efficiency was reached 40, 65 and 80% at concentrations 25, 50 and 100%, respectively. The results clearly indicated that the fungicide even at the recommended dose didn't completely inhibit the disease. There have been few studies to evaluate the potential of silver nanoparticles synthesized using biogenic methods for the control of phytopathogenic fungi in agriculture and pests. Silver nanoparticles were tested *in vitro* against *Alternaria alternata*, *P. digitatum* and *Alternaria citri* isolated from citrus fruits (Abdelmalek and Salaheldin, 2016), this nanoparticle at 150 mg l^{-1} showed good antifungal efficacy against the three tested pathogenic fungi. The results of this study confirmed silver nanoparticles as promising nanomaterials, even if compared to iprodione or difenoconazole fungicides at the same concentration. Qian *et al*. (2013) synthesized silver nanoparticles using the fungus *Epicoccum nigrum* and observed their activity against isolates of the pathogenic fungi *C. albicans*, *Fusarium solani*, *Sporothrix schenckii*, *Cryptococcus neoformans*, *Aspergillus flavus*, *and Aspergillus fumigatus*. Balakumaran *et al*. (2015) synthesized silver nanoparticles using the fungus *Guignardia mangiferae* and reported their potential to control the phytopathogenic fungi *Colletotrichum* sp., *Rhizoctonia solani*, and *Curvularia luna*ta. In other work, silver nanoparticles synthesized using the phytopathogenic fungus *Fusarium solani* isolated from wheat were shown to be effective for the treatment of wheat, barley, and maize seeds contaminated by different species of phytopathogenic fungi (Abd El-Aziz *et al*., 2015).

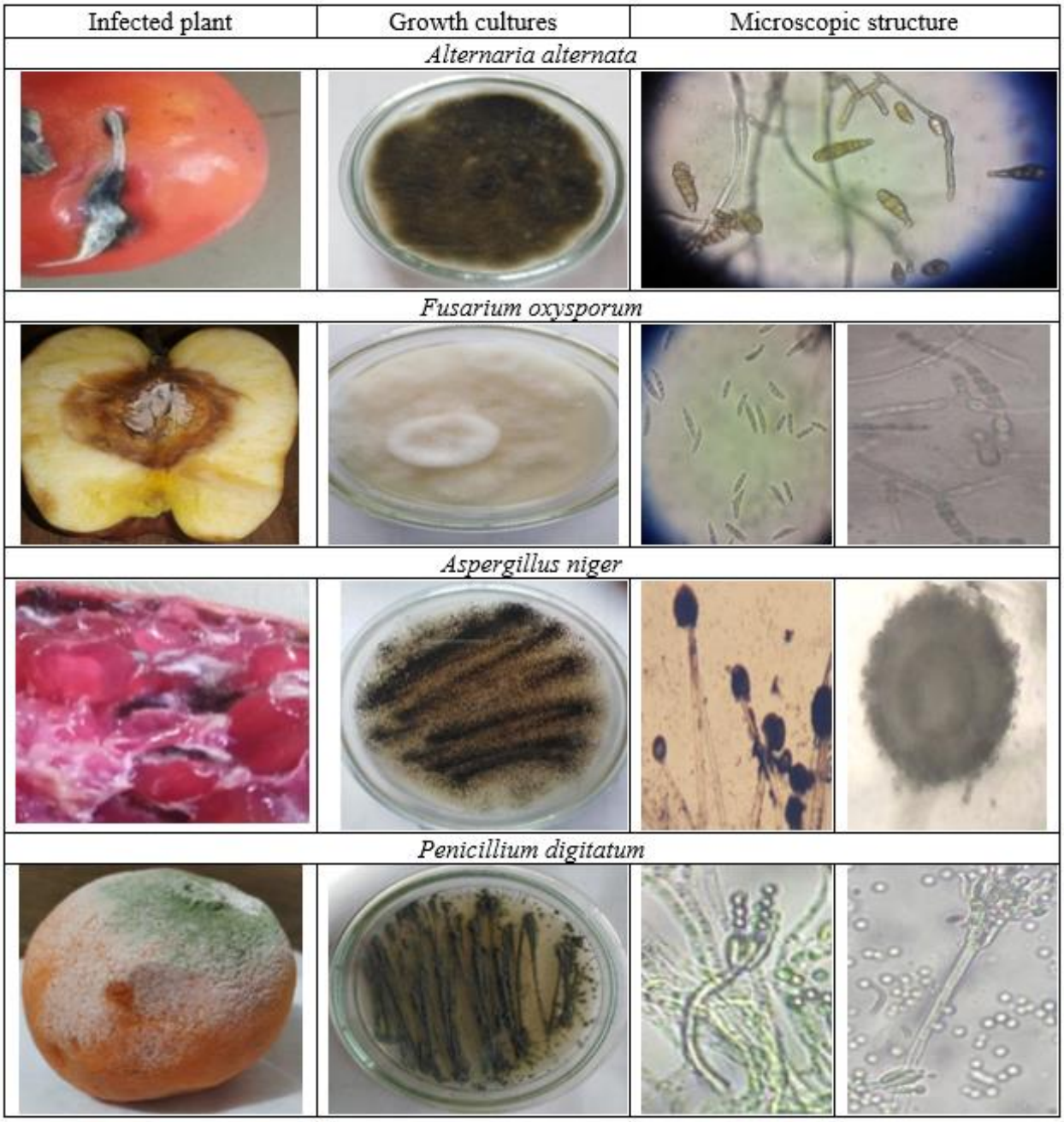

Plate 1. The microscopic structure of isolated plant pathogenic fungi. The microscopic examination observed at a magnification of 40x.

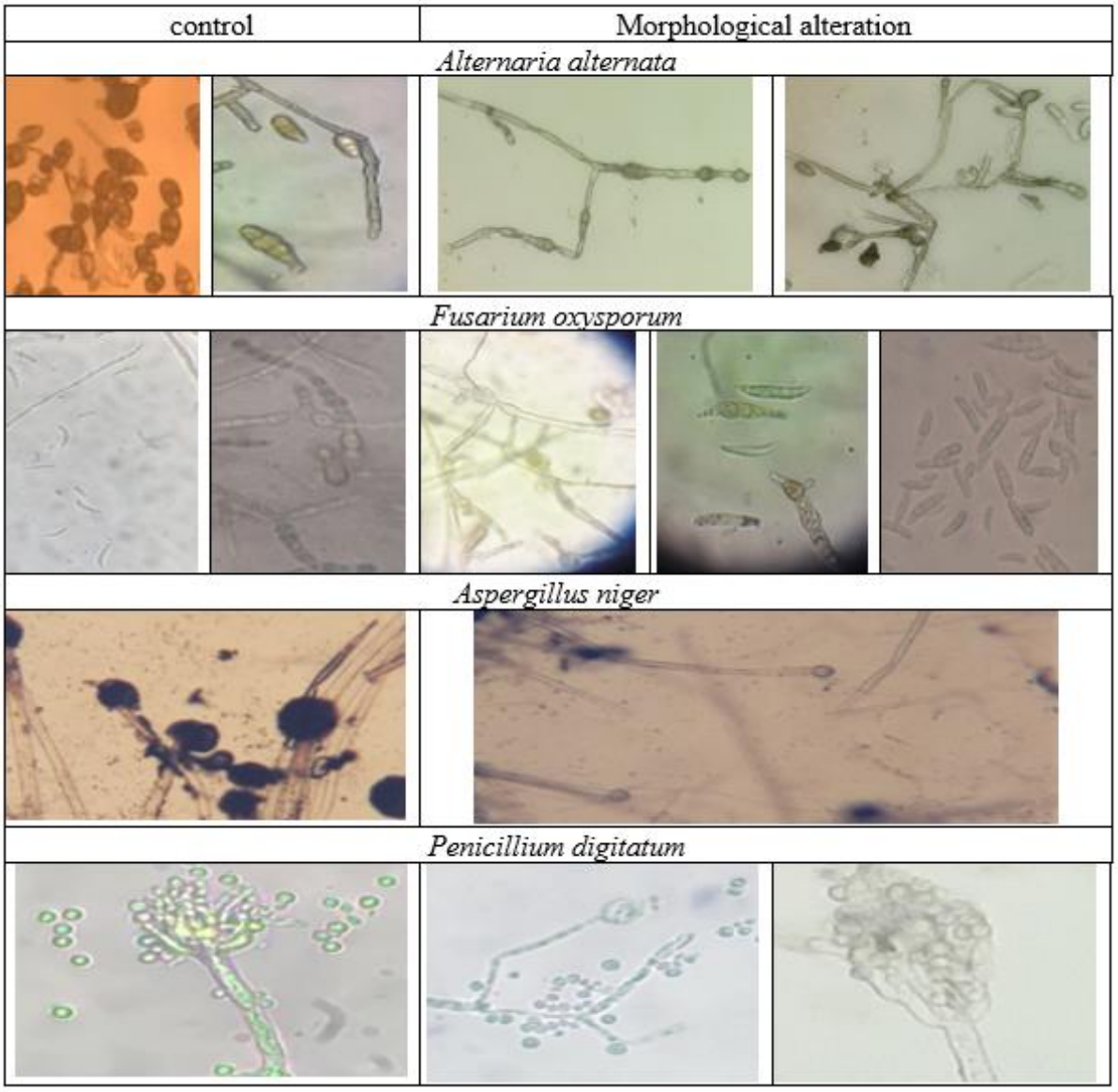

Plate 2. Morphological alteration of fungi resulted from AgNPs. The microscopic examination observed at a magnification of 10x and40x.

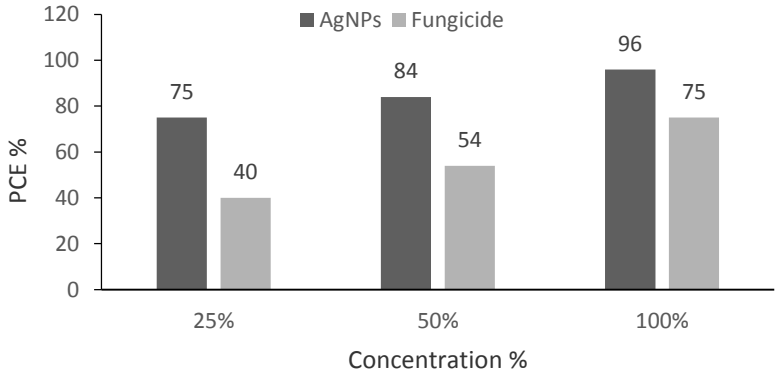

Fig. 4. percentage of control efficiency (PCE) of AgNPs and fungicide against green mould disease on orange fruits caused by *P. digitatum*.

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ARABIC SUMMARY

كفاءة نشاط جسيمات الفضة النانوية المنتجة بواسطة الفطرة الداخلية فيوزاريم كلاميدوسبورم ف٢٥ ضد الفطريات الممرضة للنباتات ما بعد الحصاد

مى احمد يونس الخواجة

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تم عزل عشرة عزلات فطرية من نبات الكالوتروبس بروسيرا. وقد اظهرت النتائج ان عزلة واحدة قادرة على انتاج جسيمات الفضة المتناهية الصغر. تم تعريف العزلة الفطرية عن طريق تعريف التسلسل الجيني وتبين انها فيوزاريوم كلاميدوسبورم ف٢٥ كما تم دراسة جسيمات الفضة النانونية المنتجة منها بواسطة التحليل الطيفي للاشعة السينية و المجهر الالكتروني النافذ. تم عزل أربع عزلات فطرية ممرضة لبعض النباتات فيما بعد الحصاد وتعريفهم عن طريق الخصائص الميكرسكوبية وتبين من الفحص انهم الترناريا الترناتا ، فيوزاريوم اوكسيسبورام، اسبريجيليس نيجر ، بينيسيليوم ديجيتيتم. وقد اختبر تأثير جسيمات الفضة النانونية المنتجة من فطرة فيوزاريوم كلاميدوسبورم ف٢٥ على هذة الفطريات الممرضة. و قد اظهرت النتائج ان هذة الجسيمات لها تاثير سام على الفطريات الممرضة . وقد اختبر تاثير جسيمات الفضة المتناهية الصغرعمليا كمبيد مضاد لمرض العفن الاخضر الذى يصيب ثمار البرتقال فى مرحلة ما بعد الحصاد وتبين بالنتائج فاعليتها كمانع لحدوث المرض.