Comparing the Antifungal Potency of Silver Nanoparticles and Traditional Fungicides against *Fusarium oxysporum*

Ahmed Osman[1]; Ahmed G. El-Shamy[2]; Abo Shabana M. Abd EL-Rahman[1]; Laila A. Reda[1]; Yousry M. Ahmed[1]; Sara Greish[1]; Mona M. Gaber[1]

[1]Plant Protection Department, Faculty of Agricultural, Suez Canal University, Ismailia, Egypt

[2]Physics Department, Faculty of science, Suez Canal University, Ismailia, Egypt

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Abstract: One of the major issues facing the world today is the control of phytopathogenic fungus. One of the principal fungal diseases affecting agricultural foodstuffs in terms of crop production and monetary loss is the *Fusarium* genus. A novel technology with application potential in many industries, including agriculture, is nanotechnology. This study focused on determining potential effects of silver nanoparticles (AgNPs) compared with two traditional fungicides namely, thyophenate-methyl (Topsin-M®) and ethylene-bis-dithio carbamate (Mancozeb®) against phytopathogenic *Fusarium oxysporum*. AgNPs and Topsin-M® both showed comparable outcomes for inhibiting mycelial growth, with corresponding percentages of 86.3% and 92.3%.This study demonstrated the promising effectiveness of AgNPs in controlling phytopathogenic fungi.

Keywords: Silver nanoparticles, Topsin-M®, Mancozeb®, Antifungal effect, Radial growth

INTRODUCTION

Plant diseases continue to cause an annual, progressive decline in agricultural product productivity and quality throughout the world. A variety of pathogenic microorganisms found in the environment and the soil rhizosphere cause diseases in plants. One of the most major and pervasive wilt pathogens that affect agricultural yields is *Fusarium* (Çolak and Bicici, 2013). The soilborne pathogen *F. oxysporum* is thought to be a constraint on tomato production because it damages the roots of the plant (McGovern, 2015; Jabnoun-Khiareddine *et al*., 2019). Plant fungal diseases control depends mainly on chemical pesticides and there is different types of chemicals fungicides have been applied to eliminate fungal contamination which the majority of them are accountable for causing environmental pollution and health hazards (Özkara and Konuk, 2016; Kumari and John, 2018). For these reasons, suitable replacements for these compounds as antifungal agents should be researched.

Recent research has examined a number of metallic nanoparticles as antibacterial agents for the management of microorganisms, including phytopathogens. Since ancient times, silver has been widely known for its antibacterial properties (Kasprowicz *et al*., 2010; Tien *et al*., 2018). Additionally, through enhancing antibacterial action, silver nanoparticles (AgNPs) prevent the development of pathogen resistance (Loo *et al*., 2018). The antibacterial activity of nanoparticles (metal/metal oxide, metalloid, and non-metal nanomaterials) on test pathogens is reportedly based on size- and dose-dependent mechanisms (Raghupathi *et al*., 2011). Silver nanoparticles (AgNPs) range from 1 nm and 100 nm in size and are widely used in industry due to their anti-microbial properties. Common applications include the use of AgNPs for antimicrobial coatings on textiles and electronics to control bacteria (Rai *et al*., 2009). AgNPs' specific anti-microbial mechanism is still not entirely known, although studies indicate there are a variety of ways nanoparticles cause damage.

The size of nanoparticles can affect microstructure and mechanical properties (Li and Chan2015). AgNPs' specific anti-microbial mechanism is still not entirely known, although studies indicate there are a variety of ways nanoparticles cause damage. Nanoparticles have a propensity to interfere with a wide range of biological functions, including microorganism cell membrane construction and function (Pal *et al*., 2007). Additionally, AgNPs block the expression of proteins linked to ATP synthesis (Yamanaka *et al*., 2005). Also Nanoparticles can improve the ability of roots and leaves to fix nitrogen and perform photosynthesis, respectively, where they can promote energy use and conversion efficiency (El-Batal *et al*., 2016; Sharaf *et al*., 2016). This work aimed to check the antifungal activity of AgNPs against *F. oxysporum* and compared it with two conventional fungicides, thyophenate-methyl (Topsin-M®) and ethylene-bis-dithio carbamate (Mancozeb®).

MATERIALS AND METHODS

Synthesis of AgNPs

AgNPs were made utilizing the chemical reduction approach, which involves reducing silver nitrate ($AgNO_3$) with ice-cold sodium borohydride ($NaBH_4$). We bought $AgNO_3$ and $NaBH_4$ from Sigma Ltd. As a precursor for metal salts, $AgNO_3$ solution (0.001 M) was used, and $NaBH_4$ (0.002 M) served as a reducing and stabilizing agent.

Add 30 mL of 0.002M sodium borohydride ($NaBH_4$) to an Erlenmeyer flask. Add a magnetic stir bar and place the flask in an ice bath on a stir plate. Stir and cool the liquid for about 20 minutes.

Drip 2 mL of 0.001M silver nitrate ($AgNO_3$) into the stirring NaBH4 solution at approximately 1 drop per second. Stop stirring as soon as all of the $AgNO_3$ is added.

Characterization of AgNPs

Ultraviolet- visible spectroscopy (UV-Vis)

The plasmonic surface resonance of AgNPs, which is characterized by absorbance at wavelengths ranging from 200 to 800 nm, was detected using UV-Vis spectroscopy. A quartz cuvette was filled with about 300 µL of colloidal AgNPs, and the absorbance was assessed using a JASCO V-570 UV/VIS/NIR double beam spectrophotometer.

Transmission Electron Microscopy (TEM)

The surface shape and particle size of AgNPs were determined with the aid of a transmission electron microscopy (TEM) model (JEOL-JEM-1230). The TEM ran between 80 and 100 kV.

Zeta potential

A dynamic light scattering (DLS) device from HORIBA, the Zeta sizer nano series, was used to identify the surface charges of nanoparticles. Prior to the experiments, a sample of nanoparticles was sonicated for 10 minutes after being diluted with deionized water.

Source of fungal strains

F. oxysporum was obtained from the plant botany department, faculty of agricultural, Suez Canal University and maintained on Potato Dextrose Agar (PDA) media

Determination of the Antifungal Activity of AgNPs and traditional fungicide

The antifungal effects of AgNPs and two conventional fungicides namely, thyophenate-methyl 70% wettable powder (Topsin-M®) and ethylene-bis-dithiocarbamate wettawble powder (Mancozeb®) was evaluated against the phytopathogenic fungi *F. oxysporum*. Different concentrations (5, 10, 25, 50 and 100 ppm) of the AgNPs and the conventional fungicides were prepared in sterile potato dextrose agar media at 45°C. After that, the media were poured in Petri dishes. Subsequently, the pathogenic fungi were inoculated from a culture of 7 days old and placed in the center of the Petri dish. They were incubated at 25°C for 7 days. Potato dextrose agar media without the addition of the AgNPs was used as a control treatment. Each treatment was performed in triplicate. Diameter of mycelial growth *in vitro* was measured at 3, 5 and 7 days after the start of the experiment. Radial growth was converted into percent inhibition by using following formula given by (Da Silva Bomfim *et al.,* 2015).

$$\text{Percent growth inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Radial growth in control plate (mm)

T = Radial growth in the treated plate (mm)

Statistical analysis:

Percentages inhibition of mycelial growth were plotted against concentrations as log/probit regression lines, the values of EC_{50}, EC_{25} and EC_{10} values as well as the slope of the toxicity lines were calculated using Ld-p Line® software. Duncan's multiple range tests ($p \leq 0.05$) was used to compare the means of different treatments using COSTAT program.

RESULTS AND DISCUSSION

Characterization of the synthesized AgNPs

UV-Vis spectroscopy

The absorbance spectra of AgNPs stabilised with borohydride is shown in Figure (1). The spectra has a prominent absorption peak at 425 nm, which is characteristic of AgNPs. The combined excitation of all the free electrons in the particles led to this peak (Yusuf, 2019). Only nanoscale particles, which are smaller than the wavelength of the incident light, are consistent with this (Solomon *et al.,* 2007; Yusuf, 2019). Widening of the observed peak indicated the development of AgNPs of various sizes. This was additionally noted for AgNPs produced using the chemical reduction approach (Quintero-quiroz *et al.,* 2019).

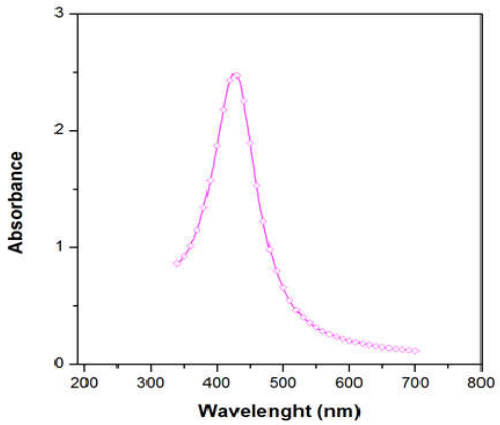

Fig. (1): UV-Vis Absorption spectrum of AgNPs solution

TEM analysis

TEM pictures were taken to gather more details regarding the size and shape of the produced AgNPs. AgNPs were depicted in TEM micrographs as being spherical with uniform borders (Fig. 2). Other studies obtained the same spherical shape of nanoparticles synthesized by the means of chemical reduction methods (Besenhard *et al*., 2018; Quintero-Quiroz *et al*., 2019). The average size was 8.52 nm, and the size distribution spanned from 6 to 18 nm (Fig. 3). The UV-Vis spectrum also demonstrated this variation in size distribution.

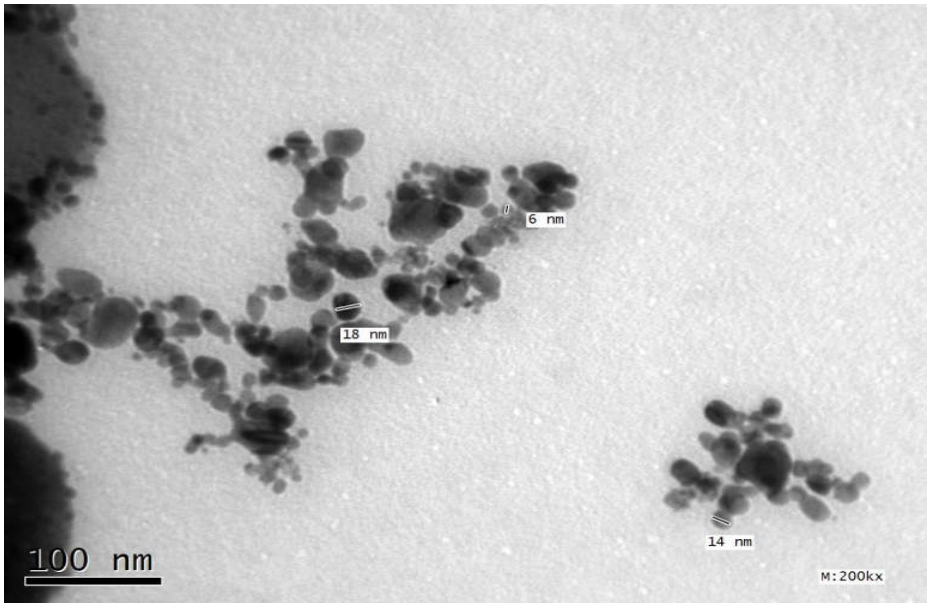

Fig. (2): TEM micrograph showing AgNPs at 200 Kx magnification

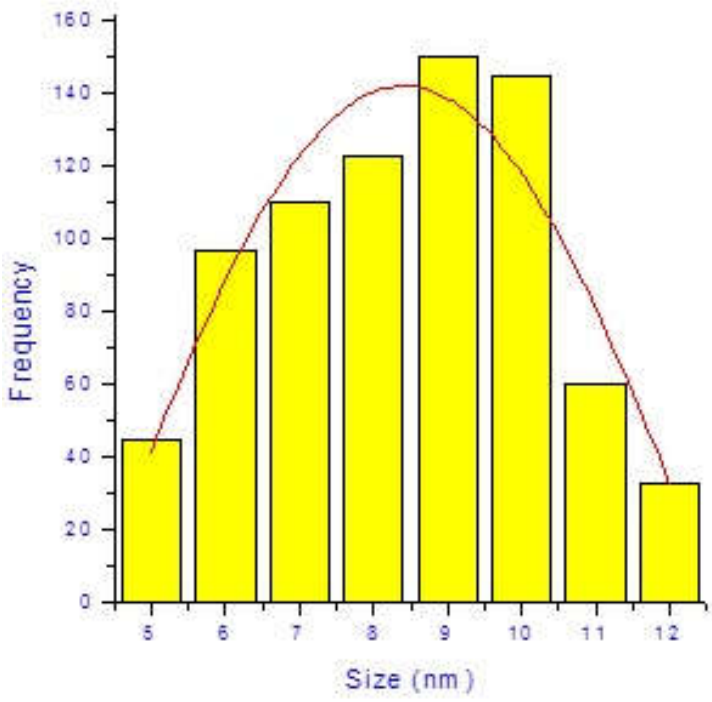

Fig. (3): Size distribution of AgNPs

Zeta (ζ)-potential

The surface charge, colloidal stability, and dispersion of the produced AgNPs when suspended in a solution were all described using the zeta ()-potential (Saxena and Shaikh, 2021). Fig. (4) Shows the value of zeta potential of AgNPs which was equal to -11.1 mV at pH=7. The value of zeta potential reported in the present study is moderate which indicated a relatively stable colloidal suspension of the synthesized AgNPs (Feng *et al*., 2022). This negative value may be ascribed to the presence of borohydride on the surface of AgNPs.

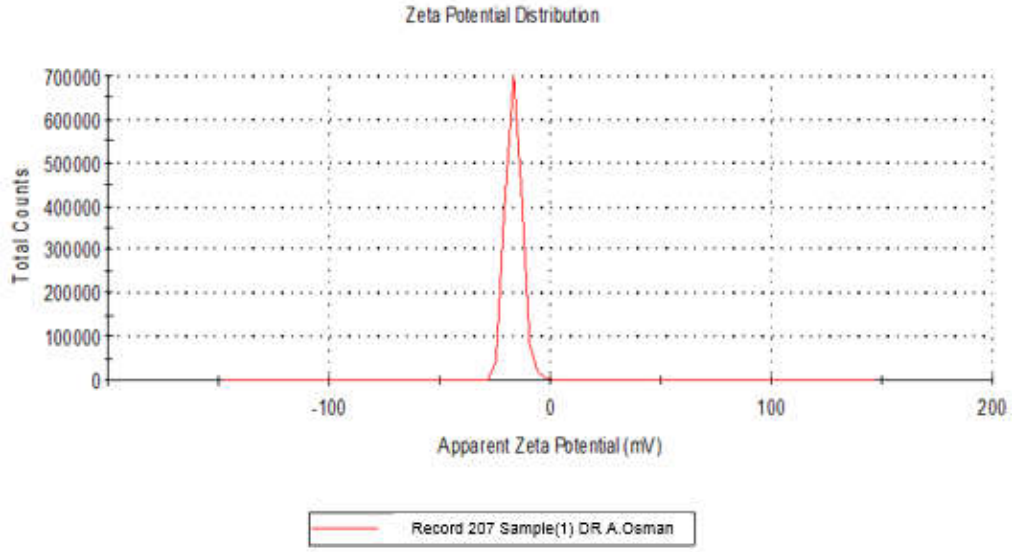

Fig. (4): Zeta potential

The inhibitory effect of AgNPs and two fungicides on the growth of *F. oxysporum*

Result in (Table 1) shows that all treatments significantly inhibition radial growth of *F. oxysporum*. Inhibition rate percentage in all treatment increase by increasing in concentration and exposure time. The highest level of inhibition was observed with Topsin-M® at a concentration of 100 ppm after 7 days followed by AgNPs with the same condition and its inhibition percentage were 92.3% and 86.3% respectively and there are a significantly differ observed between them , While the lowest level of inhibition was observed with Mancozeb® at a concentration of 5 ppm after 3days and, Its inhibition percentage was 27.4% which have not significantly differ from the next level of tested concentration at the same time.(Table 2) shows that the Comparative toxicity of AgNPs, Topsin-M® and Mancozeb® against *F. oxysporum* at a medium lethal concentration (EC_{50} level). The value of EC_{50} obtained from AgNPs after 3, 5 and 7 days was lowest than those obtained from Topsin-M® which was 16.80ppm ranged from (11.02-24.01), 11.32 ranged from (6.85-16.14) and 7.45 ranged for 4.11-10.92 for AgNPs after 3,5and7days of treatment respectively and 6.64 ppm ranged from 1.92-11.80, 3.60 ranged from .7-7.19 and 2.34 ranged from 0.86-4.44 for Topsin-M® after 3,5and7days of treatment respectively.

The results obtained that Topsin-M® was found to be highly effective for managing *F. oxysporum* after the 3, 5 and 7 days of treatment.

Boxi *et al*. (2016) reported that the fungicidal effect of the nanoparticles for *Venturia inaequalis* and *F. solani* is results of the presence of Ag+ that acts in the formation of stable Ag-S and disulfide bonds in cellular protein, which leads to cell damage. Villamizar-Gallardo *et al*. (2016) found that *F. solani* shows tolerance to AgNPs as a result of their complex multicellular organization and that may be the reason Topsin-M® was more effective than AgNPs in the present study.

Different parameters, such as particle size and particle concentration, which affect the efficiency of the fungicidal effect, showed that small sized particles (12.7 nm) are very effective in preventing the fungal growth. It is well known that silver nanoparticles are highly toxic to microorganisms. Silver nanoparticles have been known to have inhibitory and antimicrobial effects and thus we extend its application as an antimicrobial agent. Recent studies showed that nanoparticle size, shape, and core composition are strong determinants of the inhibitory action of AgNPs on fungal phytopathogens (Shafaghat, 2015; Kim *et al*., 2009). For example, Kotzybik *et al*. (2016) described a change in antifungal activity according to the size of nanoparticles. In this case, the particles of smaller sizes had in general and over the whole experimental setups a more pronounced influence on the inhibition of fugal in comparison to particles exhibiting larger sizes.

For many decades, silver (Ag+) has been studied in its use in disinfection processes against several pathogenic microorganisms, since it has environmentally friendly characteristics and a powerful

antimicrobial capacity. (Kim *et al*., 2012) evaluated the use of AgNPs at concentrations of 10, 25, 50 and 100 ppm against 18 phytopathogenic fungi on potato dextrose agar.The authors found a dose-dependent antifungal effect, observing 100% growth inhibition of the fungi *Alternaria brassicola*, *Cylindrocarpo destrutans*, *Fusarium* sp, *Pythium aphanidermatum* and *Pythium spinosum* at the highest concentration (Kim *et al*., 2012). The AgNPs mode of antifungal action can be through morphological, structural and physiological changes, altering the cell membrane, affecting the fungus hyphae and conidia, producing reactive oxygen species (ROS) that cause damage to proteins, lipids and nucleic acids (Ouda, 2014).

Dasgupta and Ramalingam (2016) showed that the main mechanism of nano-silver formulations is ROS generation and increase in membrane permeabilization. In addition, they can have a detrimental effect on sugar, proteins, n-acetyl glucosamine and lipids of the cellular filtrate and cell wall components of phytopathogens. They have also been associated with the union and penetration in the cell membrane to kill spores, although this mechanism is not completely known (Pietrzak *et al.,* 2015).

Kumari *et al*. (2019) demonstrated that after membrane disintegrate, the cells lead to death by loss of membrane structure and osmotic balance in AgNP treated fungus; additionally, they observed collapse of redox homeostasis, antioxidant machinery, cellular virulence and damage of cell wall and membrane by disrupting osmotic balance as major mechanisms. The results of this investigation exhibit the considerable difference in mycelial growth of *F. oxysporum* as a response to each fungicide and AgNPs. There was a significant reduction in the growth percent of *F. oxysporum* as a response to each fungicide and AgNPs.

Table (1): Effect of different concentrations of AgNPs and two traditional fungicides on inhibition growth percent of *F. oxysporum* after 3, 5 and 7 days of treatment

Treatment	Inhibition rate (%)														
	3 days					5 days					7 days				
	Concentration ppm														
	5	10	25	50	100	5	10	25	50	100	5	10	25	50	100
AgNPs	36.6^{p}	42.3^{o}	51.7^{lm}	66.5^{gh}	71.2^{f}	42.8^{o}	48.3^{n}	54.5^{kl}	65.5^{h}	81.9^{c}	47.9^{n}	52.2^{lm}	64.1^{hi}	71.3^{f}	86.3^{b}
Topsin-M®	49.4^{mn}	55.1^{k}	57.2^{jk}	63.7^{hi}	77.9^{d}	57.1^{jk}	58.7^{j}	62.3^{i}	69.6^{f}	83.9^{bc}	61.5^{i}	68.7^{fg}	75.2^{e}	81.2^{c}	92.3^{a}
Mancozeb®	27.4^{q}	29.7^{q}	33.8^{p}	44.2^{o}	58.1^{j}	28.1^{q}	33.6^{o}	36.2^{p}	48.8^{n}	63.5^{hi}	34.2^{p}	36.1^{p}	41.4^{o}	52^{lm}	70.1^{f}
Control	0^{r}	0^{r}	0^{r}	0^{r}	0^{r}	0^{r}	0^{r}	0^{r}	0^{r}	0^{r}	0^{r}	0^{r}	0^{r}	0^{r}	0^{r}

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range tests ($p \leq 0.05$)
Means followed by the different letter(s) are significantly different according to Duncan's multiple range tests ($p \leq 0.05$)

Table (2): Comparative toxicity of AgNPs, Topsin-M® and Mancozeb® against *F. oxysporum* after 3, 5 and 7days treatments

Compound	Exposure time (Days)	Line equation Regression of probit (y) on log concentration (x)	Slope (b)	Concentration (ppm)			
				EC_{50}	EC_{50} limit	EC_{25}	EC_{25} limit
AgNPs	3	y = 4.104 + 0.729 x	0.732 +/-0.12	16.8	11.02-24.01	2.02	0.56-3.97
Topsin-M®	3	y = 4.555 + 0.533 x	0.518 +/-0.12	6.64	1.92-11.80	0.33	0.18-1.34
Mancozeb®	3	y = 3.882 + 0.601 x	0.605 +/-0.12	71.50	43.76-168.15	5.49	1.72-9.71
AgNPs	5	y = 4.163 + 0.792 x	0.764 +/-0.12	11.32	6.85-16.14	1.48	0.39-3.04
Topsin-M®	5	y = 4.655 + 0.578 x	0.542 +/-0.12	3.60	0.7-7.19	0.28	0.08-0.90
Mancozeb®	5	y = 3.885 + 0.665 x	0.673 +/-0.12	46.88	31.50-85.36	4.67	1.63-8.11
AgNPs	7	y = 4.256 + 0.844 x	0.812 +/-0.12	7.45	4.11-10.92	1.10	0.28-2.33
Topsin-M®	7	y = 3.66 + 1.205 x	1.205 +/-0.12	2.34	0.86-4.44	0.29	0.02-0.92
Mancozeb®	7	y = 3.855 + 0.762 x	0.673 +/-0.12	32.11	22.85-48.42	4.19	1.67-7.02

CONCLUSION

In this study, AgNPs were synthesized using $NaBH_4$ as a reducing and stabilizing agent. The synthesized AgNPs had a spherical shape with regular edges and their average size were 8.5 ± 2 nm. AgNPs significantly inhibited the mycelial growth of *Fusarium oxysporium* at all the studied concentrations and time intervals with percentages ranging from 36.6-86.3 %. This suggests that it could be used as an alternative for traditional fungicides.

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مقارنة الفعالية المضادة للفطريات لجزيئات الفضة النانوية ومبيدات الفطريات التقليدية ضد فطر فيوزاريوم اوكسيسبورم

أحمد عثمان[١]، أحمد جمال الشامى[٢]، أبو شبانة مصطفى عبدالرحمن[١]، ليلى على رضا[١]، يسرى محمد أحمد[١] سارة جريش[١]، منى جابر[١]

[١]قسم وقاية النبات-كلية الزراعة - جامعة قناة السويس - الإسماعيلية - مصر

[٢]قسم الفيزياء - كلية العلوم - جامعة قناة السويس - الإسماعيلية - مصر

أحد أهم القضايا الرئيسية التي تواجه الإنتاج الزراعي في العالم اليوم هي مكافحة الفطريات الممرضة للنبات. جنس الفيوزاريوم أحد أهم مسببات الأمراض الفطرية الرئيسية التي تصيب المواد الغذائية الزراعية من حيث إنتاج المحاصيل والخسارة المالية. تعتبر تكنولوجيا النانو من الأنماط الحديثة للتكنولوجيا ذات الإمكانات التطبيقية في العديد من الصناعات، بما في ذلك الزراعة. ركزت هذه الدراسة على تحديد الفاعلية المضادة للفطريات لجزيئات الفضة النانوية (AgNPs) مقارنة باثنين من مبيدات الفطريات التقليدية هما ثيوفينات - ميثيل (توبسين-ام) وإيثيلين - بيس - ديثيوكاربامات (مانكوزيب) ضد فطر فيوزاريوم اوكسيسبورم الممرض للنبات. أظهر كل من جزيئات الفضة النانوية و(توبسين- ام) نتائج مماثلة لتثبيط نمو الفطريات، بنسب مقابلة تبلغ ٨٦.٣٪ و ٩٢.٣٪. أظهرت هذه الدراسة الفعالية الواعدة لجزيئات الفضة النانوية في السيطرة على الفطريات الممرضة للنبات.