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المجلة العراقية لبحوث السوق
وحماية المستهلك

EVALUATING THE EFFICIENCY OF SOME NANOPARTICALS IN INHIBITING THE GROWTH OF *SCLEROTINIA SCLEROTIORUM* (LIB) DEBARY, THE CAUSE OF EGGPLANT WHITE MOLD DISEASE IN LABORATORY CONDITIONS

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ABSTRACT

This experiment was applied in the Plant Diseases Laboratory/ Postgraduate Studies/ Department of Plant Protection/ College of Agricultural Engineering Sciences/ University of Baghdad. Four isolates of *Sclerotinia sclerotiorum* (Lib) DeBary were collected from infected eggplant grown in different greenhouses in Baghdad Governorate. The pathogenicity of isolates was studied in greenhouse conditions, and the results showed that all isolates were pathogenic. All isolates were identified molecularly using the specialized primers SSFWD, the isolate SR7 was registered at the National Center for Biotechnology Information (NCBI) under accession numbers OR711897.1. The enzymatic activity of the isolates was tested, and isolate SR7 was the most active. The effect of silica, copper and magnesium oxides as nanoparticales on the growth of fungal hyphae of *Sclerotinia sclerotiorum* (Lib) DeBary was tested at two concentrations of 0.2 and 0.5 g/L in laboratory conditions. The nanoparticale copper oxide was superior in both concentrations with an inhibition rate of 100%, with significant differences from the nanoparticale silica and magnesium oxides, where the inhibition rate in the first concentration reached 36.3 and 50.5%, respectively, while in the second concentration it reached 61.1 and 64.2%, respectively.

Key words: *Sclerotinia sclerotiorum*, ,Pathogenicity, Nanoparticales Nano copper oxide.

[*] **This article is taken from the doctoral dissertation of the first researcher.**

تقييم كفاءة بعض المواد النانوية في تثبيط نمو الفطر *Sclerotinia sclerotiorum (Lib) DeBary* مسبب مرض العفن الابيض على نبات الباذنجان في ظروف المختبر

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الخلاصة

طبقت هذه التجربة في مختبر امراض النبات/ الدراسات العليا / قسم وقاية النبات /كلية علوم الهندسة الزراعية /جامعة بغداد. جمعت اربع عزلات من الفطر *Sclerotinia sclerotiorum* (Lib) DeBary من نباتات باذنجان مصابة مزروعة في بيوت بلاستيكية مختلفة في محافظة بغداد. درست امراضية كل العزلات المختبرة في ظروف البيت البلاستيكي واظهرت النتائج ان جميع العزلات كانت ممرضة. شخصت جميع العزلات جزيئيا باستخدام البرايمر المتخصص SSFWD وسجلت العزلة SR7 في المركز الوطني لمعلومات التقانة الحيوية NCBI تحت رقم انضمام OR711897.1 .درس النشاط الانزيمي للعزلات وكانت العزلة SR7 اكثرها نشاطا. اختبر تأثير كل من اكاسيد السيلكا والنحاس والمغنيسيوم النانوية في نمو الخيوط الفطرية للمسبب *Sclerotinia sclerotiorum* (Lib) DeBary بتركيزين 0.2 و0.5 غم /لتر في ظروف المختبر. تفوق اوكسيد النحاس النانوي في كلا التركيزين بنسبة تثبيط 100% وبفروق معنوية عن اكاسيد السيلكا والمغنيسيوم النانوية والتي بلغت نسبة التثبيط في التركيز الاول 36.3 و50.5% على التتابع بينما بلغت في التركيز الثاني 61.1 و 64.2 % على التتابع.

الكلمات المفتاحية: *Sclerotinia sclerotiorum* , الامراضية , الجسيمات النانوية , اوكسيد النحاس النانوي.

INTRODUCTION

The fungus *Sclerotinia sclerotiorum* (Lib) DeBary is a non-specialized soil pathogen with a wide family range, infecting more than 600 plant species **(Matny *et al.*, 2014؛ O'Sullivan *et al*, 2021; Hossain *et al.*, 2023)**. It is also considered a facultative parasitic necrotrophic fungus **(Harel *et al.*, 2006; Rafiqi *et al.*, 2012)**. This fungus has a set of symptoms that vary depending on the plant host, but in general it is characterized by the appearance of watery areas that turn into irregularly shaped brown spots on the various parts of the plant, which are later covered with white, cottony fungal hyphae. Then a number of sclerotia are formed, which are a distinctive diagnostic sign of infection with the pathogen. Patients **(Dilantha *et al.*, 2004)**, which maintain their vitality for more than ten years **(Liu *et al.*, 2017).** This fungus is an important pathogen on eggplant **(Samir & Neamah, 2013)**. It also causes plants to wilt, which leads to a significant loss in yield **(Hami *et al.*, 2021).**

Pest management depends largely on the use of chemical pesticides, such as insecticides, fungicides, herbicides, and others. Secondary compounds of plant extracts are also a common method of combating pathogens **(Karim *et al*, 2021).** Chemical pesticides are characterized by several characteristics: they are well available, highly efficient, and quickly effective **(Stephenson, 2001; Wang, 2019)**. Pesticides were also used in conjunction with some other control methods, such as biological control, which led to a reduction in the incidence of pathogens **(Al-Amiri & Al-Badour, 2016)**. However, despite this, it has many drawbacks, such as its harmful effect on non-target organisms, the development of resistant strains of pathogens, its toxicity, and its danger to human and animal health. Therefore, the need has emerged to find less harmful alternatives to preserve the environment and public health **(Arslan *et al.*, 2009; Heydari & Pessarakli , 2010; Atiq *et al.*, 2020)**, and thus many chemical pesticides were banned **(Krasnow, 2022)**.

Nanoparticales have many benefits and their application requires good knowledge of several sciences, as they are used in various fields such as agriculture because of their physical and chemical properties that appear due to reducing the size of particles in current formulations of agricultural chemicals to the nanoscale. **(Prasad, 1914; Khan *et al*., 2016)**. Nanoparticles (NPs) have attracted great interest in various fields due to their antimicrobial activity **(Sadek *et al*., 2022; Kamel *et al*., 2022)**, It also stimulates host-resistant plants by increasing the activity of resistance enzymes such as catalase and peroxidase, and thus it has become possible to reduce the use of chemical pesticides by using nanoparticales in agriculture **(Arora & Padau, 2010; Tomah, 2020)**. Nanoparticales have also been used to prepare antioxidants that are safe for human health **(Hamdia & Ahmad, 2023)**.

Nanotechnology has advanced significantly and led to the development of new concepts and products with enormous potential to manage agricultural pest problems by controlling the release of agricultural chemicals **(Sinha *et al*., 2017; Balaure *et al*., 2017; Hayles *et al*., 2017)**. The importance of green synthesis for the synthesis of nanoparticles was mentioned, which provided a simple, fast and effective method such as copper and silver **(Ponmurugan *et al*., 2016؛Al-Juboury *et al*., 2022; Khansaa *et al*., 2023)**. Silver nanoparticles have been used to inhibit many fungi **(Mishra & Sharma, 2015)**. Nanoparticles also have the ability to be applied directly to plant seeds, leaves or roots to protect them from pests and pathogens. Extensive research has been conducted on metal nanoparticles such as silver, copper, zinc oxide, and titanium dioxide for their anti-plant pathogen properties such as bacteria, fungi, and others **(Kah & Hofmann, 2014; Kim *et al*., 2018)**. Thus, nanoparticales may reduce the amount of pesticides used to combat plant diseases **(Singh *et al*., 2015)**. Nanoalgae were used to combat the pathogenic fungus *S. sclerotiorum* **(Hamoudi, 2020)**. Nano-magnesium oxide and nano silicon is also a good control agent against a number of pathogens **(Hateem & Khazal, 2021; Abdul-Karim & Aljarah, 2023)** .

study aims to evaluate the efficiency of some nanoparticales in combating the pathogenic fungus *Sclerotinia sclerotiorum* (Lib) DeBary, which causes white mold disease on eggplant crops under laboratory conditions.

MATERIALS AND METHODS

Isolation of *Sclerotinia sclerotiorum* (Lib) DeBary

Eggplant showing symptoms of white mold disease caused by *S. sclerotiorum* (Lib) DeBary were collected from greenhouses in different areas of Baghdad Governorate. The sclerotia formed inside the stems of infected plants were collected and surface sterilized with sodium hypochlorate solution (chlorine 1%) for 2-3 minutes, then washed twice with sterile distilled water **(Kareem *et al.*, 2020)**, dried on sterile filter paper, and then planted in 9 cm diameter plastic Petri dishes prepared with sterile Potato Dextrose Agar (PDA) culture medium. The antibiotic Streptomycin sulfate 50 g/L PDA was added to it **(Abdul-karim &Aljarah, 2023)**, and the dishes were incubated at 20°C until sclerotia formed, then they were cultured again in the same way. The isolates were purified after 4-5 days by transferring part of the edges of the fungal growths of the colony to Petri dish (9cm diameter) containing the PDA culture medium. The sclerotia of the isolates were preserved until they were used in subsequent

experiments for the study. Four isolates were obtained, given the codes SR5, SR6, SR7, and SR8, respectively.

Molecular diagnosis of *S. sclerotiorum* (Lib) DeBary:

Fungal isolates were identified molecularly using a specific primer pair forward SSFWD-F-5′CTGCTCTTCGGGGCCTTGTATGC3′ and reverse SSREV-R-5′ TGACATGGACTCAATACCAAGCTG3′ **(Freeman *et al.*, 2002)**. DNA was extracted by taking 1 g of isolates of the studied fungus after growing it in Petri dishes containing PDA medium. a premix PCR kit from Bioneer Company has been used **(Hamdia, 2014; Hamdia *et al.* ,2020)**. Because SR7 isolate is the most pathogenic, it was selected for sequencing by Bioneer Company Corporation.

pathogenicity Test of *S. sclerotiorum* (Lib) DeBary Isolates

In This experiment A mixture of mixed soil and peat moss 2:1 was used after sterilizing with an autoclave (heat 121°C and pressure 1.5 kg/cm2) for an hour and re-sterilized after 24 hours **(Yasir & Almaliky, 2023)**. a plastic pots (2 kg) was used, planted with seedlings of the Jawaher variety, one month old. In each pot, two plants were grown, with four replicates per and placed inside the greenhouse with the necessary agricultural operations. Isolates SR5,SR6,SR7, and SR8 were used to inoculation eggplant by making two holes approximately 1 cm deep for each plant and placing a sclerotia in each hole **(Al-Jarrah, 2016)**. four replicates remained without inoculation with the pathogenic fungus .the readings were recorded 30 days. The severity of disease was recorded according to the scale **(Dixon & Dodson, 1971)** includes the following:

0: No injury
1: the infection length not exceeding 6 mm
2: the infection length extending 6 mm
3: the infection length exceeding 6 mm
4: Plant death

Disease severity was calculated according to the McKinney equation:

Disease severity ={(Number of plants in 0 +0……. Number of plants in 4+4)/ Total number of plants x maximum score}x100

Molecular diagnosis of *S. sclerotiorum* (Lib) DeBary:

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Detection of the ability of *S. sclerotiorum* (Lib) DeBary fungus isolates to secrete some enzymes:

This experiment was conducted to qualitatively detect the enzymatic activity of *S. sclerotiorum* (Lib) DeBary in laboratory conditions. the medium was prepared consisting of the following materials (g/liter of distilled water): NaNo3, 2.0: K2HPO4, 1.0: MgSO4.7H2O, 0.5: KCL, 0.5: FeSO4, 0.01: Cellulose, 10.0: Agar, 20.0. Cellulose is the only source of carbon. All components of the medium were added gradually to sterile distilled water and dissolved using a magnetic mixer, heating at a temperature of 20°C and at PH = 6. The medium was distributed in tightly sealed 100 ml glass flasks and sterilized with an autoclave. Pour the medium into 9 cm diameter plastic Petri dishes after it has cooled. The center of each dish was inoculated with 5 mm discs from the edge of the colony of isolates SR5, SR6, SR7, and SR8, three replicates for each isolate, and placed in the incubator at a temperature of 20 °C. The growth of fungal hyphae for each replicate of the isolates was calculated 6 days after inoculation, as the growth of the isolate indicates its ability to produce the cellulase enzyme. The four isolates of the pathogenic fungus were also tested for the production of the enzyme Laccase. The same medium as before was prepared with the addition of 0.04% Guaiacol. The medium was inoculated with isolates of the pathogenic fungus after cooling it and pouring it into dishes. The replicates were examined every 24 hours and the appearance and intensity of the reddish-brown color around the colony of each isolate was recorded. Indicates the production of the laccase enzyme by the *S. sclerotiorum* (Lib) DeBary.

Testing the efficiency of nanoparticales in inhibiting the *S. sclerotiorum* (Lib) DeBary in laboratory conditions:

The effectiveness of three nanoparticales: copper, magnesium, and silica oxides, at concentrations of 0.2 and 0.5%, against the *S. sclerotiorum* (Lib) DeBary was tested using the poisoned food media method. 100 ml conical glass flasks containing 60 ml of ready-made PDA culture medium were used, which were sterilized with an autoclave. Substance concentrations were prepared by adding known weights of each substance to obtain the required concentrations before solidifying the medium in the flasks, shaking them in a regular circular motion to ensure homogeneity of the contents, then pouring them into plastic Petri dishes with a diameter of 9 cm. Treatments included the following:

1- Nano copper oxide at concentrations of 0.2 and 0.5 g/L.
2- Nano magnesium oxide at concentrations of 0.2 and 0.5 g/L.
3- Nano silica oxide at concentrations of 0.2 and 0.5 g/L.
4- Control (PDA + isolate SR7 only) .

Each dish was inoculated with a 5 mm diameter disk taken from the edge of the SR7 colony of the *S. sclerotiorum* (Lib) DeBary 5-6 days old, grown in 9 cm diameter Petri dishes containing the PDA culture medium. The disk was placed in the center of each inoculated dish. 3 replicates were made for each treatment and compared with the control treatment (pathogenic fungus only). The plates were incubated at 20°C, and the final readings were taken 7 days after inoculation, when the growth of the pathogenic fungus was complete in the control treatment. The percentage of inhibition for each treatment was calculated according to the following equation:

Inhibition% = (a-b / a) 100
a= diameter of the pathogen in the control, b= diameter of the pathogen in the treatment

RESULTS AND DISCUSSION

Isolation of *S. sclerotiorum* (Lib) DeBary

The results of isolation and phenotypic diagnosis showed the presence of four different isolates of *S. sclerotiorum* (Lib) DeBary on PDA culture medium inoculated with sclerotia taken from plants that showed symptoms of the disease, namely SR5, SR6, SR7, and SR8 figure (1). The entire plate was covered with white fungal growth four days after inoculation. The sclerotia was formed approximately 6-7 days after inoculation, which were characterized by their varying numbers, sizes, and irregular shapes depending on the isolates, in addition to their black color and the hardness of their outer shell. There is much research that has shown that isolates of the *S. sclerotiorum* (Lib) DeBary differ in phenotypic characteristics such as fungal growth rate, number and size of the sclerotia formed, and their shape **(Ali & Al-Jarah, 2018).**

Figure (1): Isolates of *S. Sclerotiorum* (Lib) DeBary.

Molecular diagnosis of *S. sclerotiorum* (Lib) DeBary:

The PCR results obtained with the specific primers SSFWD and SSREV were carefully analyzed, and the resulting product produced by the PCR length was found to be within the (300) bp range.

The genetic sequences of the SR7- SSFWD, *Sclerotinia* sp. isolate SR7-ITS were subjected to blasting using the National Center for Biotechnology Information (NCBI) and placed into the database separately, with different GenBank accession numbers (OR711897.1).

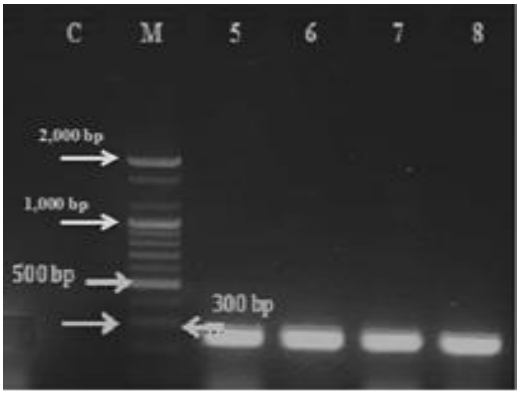

Figure (2): Electrophoresis of fungal isolates of *S. sclerotiorum* (isolate number 5-8) ~ 300 bp. PCR fragment.

pathogenicity Test of *S. sclerotiorum* (Lib) DeBary Isolates

The results showed that *S. sclerotiorum* (Lib) DeBary isolates differed in the production of funnel-shaped, light-colored apothecia in the potting soil (Figure (3). Isolate SR7 achieved the highest rate of disease severity, reaching 84.8%, This isolate did not differ significantly from SR8 76.2%. Both previous isolates differed significantly with isolates SR6 and SR5, 43.2 and 0.2%, respectively, Table (1). The difference in pathogenicity of *S. sclerotiorum* (Lib) DeBary isolates has been studied by a number of researchers **(Samir & Neamah, 2013 ؛ Ali &aljarah, 2018)**.

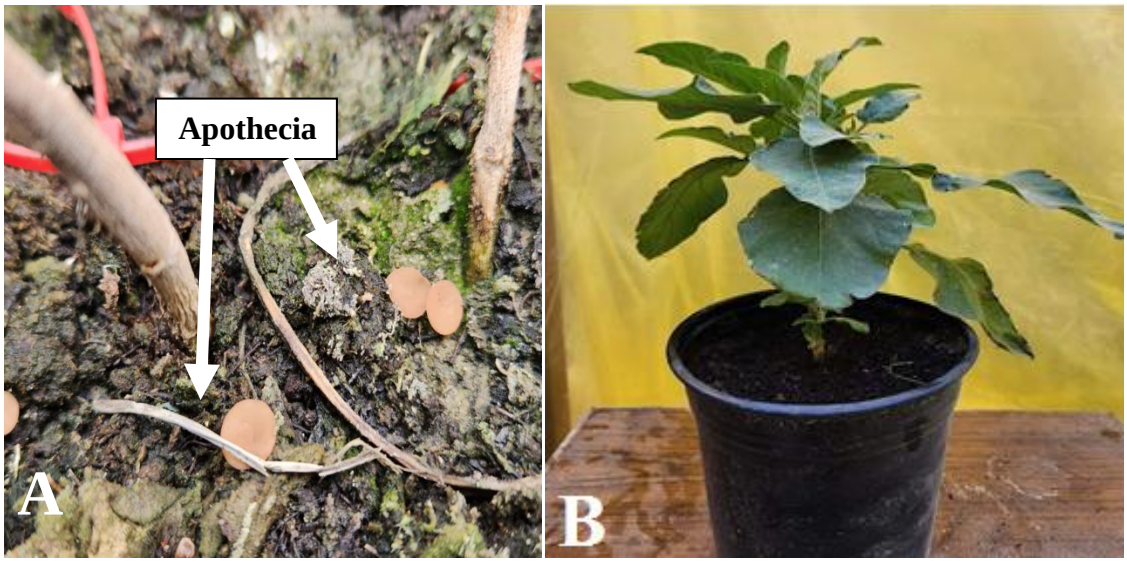

Figure (3): Pathogenicity test of *S. sclerotiorum* (Lib) DeBary isolates. (A) Aplant inoculated with a sclerotia. (B) Healthy plant (Control untreated).

Table (1): Pathogenicity of *S. sclerotiorum* (Lib) DeBary isolates under greenhouse conditions.

Isolates	disease Severity %
SR5	0.2
SR6	43.2
SR7	84.8
SR8	76.2
$LSD_{0.05}$	26.01

Detection of the ability of *S. sclerotiorum* (Lib) DeBary isolates to secrete some enzymes

The results showed the ability of all isolates to produce both Cellulase and Lacase enzymes. The formation of a reddish-brown color was observed in all isolates of the pathogenic fungus, which indicates the production of the Lacase enzyme, and it varied in the time of appearance of the color and its intensity. Isolate SR7 recorded the appearance of reddish-brown discoloration 24 hours after inoculation. Discoloration appeared in the rest of the isolates 48 hours after inoculation, with a difference in the intensity of the color, as isolate SR7 had very strong discoloration, while isolate SR5 was characterized by the least intensity of discoloration. Isolates SR6 and SR8 had strong discoloration.

All tested isolates formed colonies on the culture medium that secretes the enzyme Cellulase, and they differed in the rate of fungal growth, which indicates a difference in their ability to produce this enzyme. The highest rate of fungal growth diameter was with isolate SR7, which was 5.1 cm, it was significantly superior to all the remaining isolates, SR5, SR6, and SR8, with a fungal growth rate of 2.5, 4.23, and 4.07 cm, respectively. Isolate SR5 was characterized by the lowest fungal growth rate, and this is consistent with the results of the pathogenicity experiment. Pectin- and cellulose-degrading enzymes are important enzymes that degrade the cell walls of host plants and are considered means that help pathogenic fungi penetrate plant hosts and destroy their defenses **(Ali & Aljarah, 2018).**

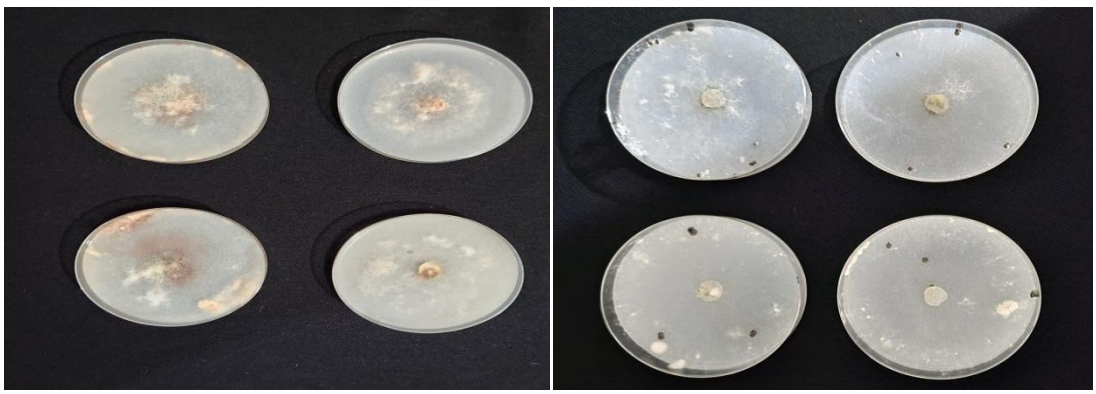

Figure (4): Ability of *S. sclerotiorum* (Lib) DeBary isolates to produce cellulase and laccase enzymes in laboratory conditions.

Table (2): Fungal growth rate of *S. sclerotiorum* (Lib) DeBary On the medium intended to detect the production of the cellulase enzyme in laboratory conditions.

Isolates	Fungal growth rate (cm)
SR5	2.5
SR6	4.23
SR7	5.1
SR8	4.7
$LSD_{0.05}$	0.47

Testing the efficiency of nanoparticales in inhibiting the *S. sclerotiorum* in laboratory conditions

The results of the examination with the Field Emission Scanning Electron Microscopy (FESEM) device showed that all materials used were less than 100 nanometers in size Figure (5).

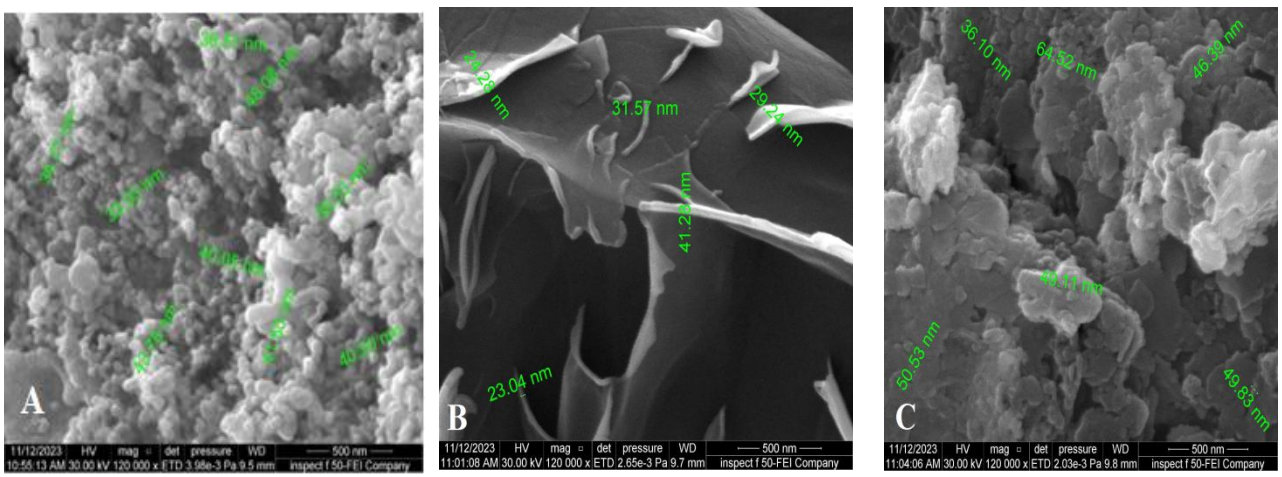

Figure (5): Nanoparticales used to inhibit the growth of *S. sclerotiorum* (Lib) DeBary in laboratory conditions. (A) Nano copper oxide (B) Nano magnesium oxide (C) Nano silica oxide.

Table (2) shows the effect of nano-silica, magnesium and copper oxides in significantly inhibiting the growth of *S. sclerotiorum* (Lib) DeBary compared to the control treatment (pathogen only on PDA medium). The highest effectiveness was recorded for nano-copper oxide, as the percentage of inhibition reached 100% at two concentrations of 0.2 and 0.5%. This was followed by treatment with magnesium oxide and nano-silica at a concentration of 0.5%, where the average percentage of inhibition reached 64.2 and 61.1%, respectively. As for the oxides that had the least effect on the growth of the fungal hyphae of the pathogen, they

were recorded in the treatment of silica oxide and nano magnesium at a concentration of 0.2%, as they reached 36.3 and 50.5%, respectively.

In general, the treatment with the best effect in inhibiting the growth of fungal hyphae of the pathogen was nano-copper oxide, then both magnesium oxide and nano-silica, with no significant differences between them, as they recorded 57.4 and 48.7%, respectively. We note from Table (2) that there are no significant differences between the two concentrations used in the experiment (0.2 and 0.5%), as the average percentage of inhibition reached 46.7 and 56.3%, respectively.

Copper is usually used in agriculture to protect plants from various pathogens as it creates highly reactive hydroxyl radicals, which may lead to damage to lipids, DNA, proteins and other biomolecules for a group of microorganisms **(Borkow & Gabbay, 2005)**. Copper nanoparticles have been used against many types of bacteria **(Mondal & Mani, 2012)**, and they have also been used as an inhibitory agent for a number of fungal pathogens. The growth of the pathogen *Botrytis cinerea* was inhibited at concentrations of copper nanoparticles of 1, 10, and 100 Pmm **(Bramhanwade *et al.* 2016 ؛Bayat *et al.*, 2021)**. Nanocopper was used against the pathogen *S. sclerotiorum* (Lib) DeBary. In an experiment, the growth of fungal hyphae was inhibited using relatively low concentrations (5-100 g/ml) compared to using regular copper sulfate **(Sadek *et al.*, 2022)**.

Table (3): Effect of some nanoparticales on the growth of fungal hyphae of *S. sclerotiorum* (Lib) DeBary in laboratory conditions.

Treatment	% Inhibition *S. sclerotiorum*		% Inhibition
	0.2 g /L	0.5 g /L	
Nano silica Oxide	36.3	61.1	48.7
Nano magnesium Oxid	50.5	64.2	57.4
Nano copper Oxide	100.0	100.0	100.0
Control	0.0	0.0	0.0
Means	46.7	56.3	9.27
$LSD_{0.05}$ concentrations	13.11		
$LSD_{0.05}$ interaction	18.55		

Nanoparticales work to improve plant characteristics. In a field experiment, the results showed that plants sprayed with nanoparticales were superior in fresh, dry and yield weight, as well as in the amount of sucrose and ascorbic acid **(Al-Juthery & Saadoun, 2018)**. The use of nanoparticles in combating some plant diseases and pests is very successful without causing any negative effects on the environment and human health. The mechanism behind the killing of pathogens by nanoparticles involves better membrane adhesion and microbial cell penetration, hence significant structural and morphological changes of the cell membrane. Nanoparticles (NPs) interact within the cell, It is distributed in the cytoplasm with respiratory

enzymes and phosphorus-containing compounds such as DNA. As a result, the replication of the microbial DNA molecule is prevented, and ultimately leads to cell death **(Ghosh & Bera, 2021).** In addition, it represents an environmentally friendly and sustainable way to control fungal infections due to its low cytotoxicity **(Sadek *et al.*, 2022)**.

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