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The Efficiency of the Reductase Enzyme in Producing Silver Nanoparticles (AgNPs) and Its Role in Inhibiting the Pathogenic fungus *Fusarium solani*

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ABSTRACT

A laboratory study was conducted during the year 2024 In the laboratories of the Plant Protection Department, College of Agriculture, Tikrit University, Iraq, Saladin The study dealt with the production of biotinylated silver nanoparticles from the *Fusarium solani*, using four parts that included the filtrate, the biomass, the hot extract, and the cold extract of the biomass. The results of the laboratory study demonstrated the formation of silver nanoparticles from the *F. solani*. This is done by detecting it by the color contrast of the leachate The color of the mushroom filtrate, the biomass, and its hot and cold extract of the biomass changed from yellow to brown. The highest absorption peaks were recorded (using the photoanalyzer) for the silver nanoparticles within the wavelengths of 350- 475 nm, as the lowest wavelength reached 350 nm for the prepared silver nanoparticles. From the hot extract, the highest wavelength reached 475 nanometers For silver nanoparticles prepared from cold extract for the fungus Fusarium solani. Electron microscope images showed the sizes of the nanoparticles synthesized by the fungus *F. solani* in semi-spherical shapes whose diameters ranged from 30-70 nanometers. This indicates the efficiency of the biosynthesis of silver particles. Laboratory results showed that no significant differences were recorded for the concentrations of silver nanoparticles in the germination rates of wheat seeds (Sham 6 variety) for all concentrations and types of silver nanoparticles prepared from all types of *F. solani* fungi studied. All concentrations and types of silver nanoparticles prepared showed an effect of inhibiting the pathogenic fungus. *F. solani* Compared to the control, the highest rate of inhibition was reached at a concentration of 1.5 mM in the filtrate of the studied mushrooms, reaching 0.64% compared to the control in which no rate of inhibition was recorded. While all concentrations and types of prepared silver nanoparticles showed the highest activity of the nitrate reductase enzyme compared to the control, the highest effectiveness was reached at the concentration of 1.5 mM in the filtrates of the studied mushrooms, reaching 0.63 units/ml compared to the control, which reached 0.055 units/ml.

Redüktaz Enziminin Gümüş Nanopartiküller (AgNP) Üretmedeki Etkinliği ve Patojenik Mantar *Fusarium solani*'yi Engellemedeki Rolü

Araştırma Makalesi

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ÖZ

2024 yılında özel laboratuvarlarda bir laboratuvar çalışması yürütülmüştür. Çalışma, süzüntü, biyokütle, sıcak özüt ve biyokütlenin soğuk özütünü içeren dört parça kullanılarak *Fusarium solani*'den biyotinlenmiş gümüş nanopartiküllerinin üretimiyle ilgili bir çalışmadır. Laboratuvar çalışmasının

Anahtar Kelimeler:
Gümüş nanopartiküller
Biyosentez
Nanoteknoloji
Fusarium solani
Buğday mahsulü

sonuçları, *F. solani*'den gümüş nanopartiküllerinin oluşumunu göstermiştir. Bu, sızıntı suyunun renk kontrastı ile tespit edilerek yapılmıştır. fungus süzüntüsünün, biyokütlenin ve biyokütlenin sıcak ve soğuk özütünün rengi sarıdan kahverengiye değişmiştir. En yüksek emilim pikleri (fotoanalizör kullanılarak) 350-475 nm dalga boylarındaki gümüş nanopartiküller için kaydedilmiş, hazırlanan gümüş nanopartiküller için en düşük dalga boyu 350 nm'ye ulaşmıştır. Süzüntünün soğuk özütünden hazırlanan gümüş nanopartiküller için sıcak özütten en yüksek dalga boyu 475 nanometreye ulaşmıştır. Elektron mikroskobu görüntüleri, *F. solani* fungus tarafından sentezlenen ve çapları 30-70 nanometre arasında değişen yarı küresel şekillerdeki nanopartiküllerin boyutlarını göstermiştir. Bu, gümüş parçacıklarının biyosentezinin verimliliğini gösteren bir ölçüttür. Laboratuvar sonuçları, çalışılan tüm *F. solani* mantar türlerinden hazırlanan tüm gümüş nanopartikül konsantrasyonları ve türleri için buğday tohumlarının (Sham 6 çeşidi) çimlenme oranlarında gümüş nanopartikül konsantrasyonları için önemli bir fark kaydedilmediğini göstermiştir. Hazırlanan tüm gümüş nanopartikül konsantrasyonları ve türleri patojenik mantarı inhibe etme etkisi göstermiştir. *F. solani* kontrol ile karşılaştırıldığında, en yüksek inhibisyon oranı, hiçbir inhibisyon oranının kaydedilmediği kontrol ile karşılaştırıldığında %0,64'e ulaşarak, çalışılan mantarların süzüntüsünde 1,5 mM'lik bir konsantrasyonda elde edilmiştir. Hazırlanan gümüş nanopartiküllerin tüm konsantrasyonları ve tipleri nitrat redüktaz enziminin kontrol ile karşılaştırıldığında en yüksek aktivitesini gösterirken, incelenen mantarların süzüntülerinde en yüksek etkinliğe 1,5 mM konsantrasyonda 0,63 ünite/ml'ye ulaşırken, kontrol ise 0,055 ünite/ml'ye ulaşmıştır.

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Introduction

Silver nanoparticles (AgNPs) have several processes in affecting the cell wall of plant pathogenic fungi through their direct effect on the protein and nucleic acids inside the pathogenic fungus, which leads to a significant growth inhibition process (Min et al., 2009). The role of fungi in the synthesis of nanoparticles, including effective silver, attracts the attention of researchers around the world because these organisms have the ability to produce nanoparticles well and also have many biological activities, as fungi can be used as an excellent source of various external enzymes that affect the synthesis of nanoparticles. Fungi have been identified as a superior alternative to bacteria and plants as nanofactories (Pradhan, 2013). Vanaja et al. (2013) indicated that the mechanisms that lead to the production of nanoparticles by fungi are limited to the use of mushroom extract, which leads to the reduction of metals, including silver, into metal particles. Thus, the synthesis of nanoparticles, and the process of ensuring the synthesis of nanoparticles takes place through several methods, including a method of biosynthesis known as green synthesis, which is characterized by being environmentally friendly. It produces materials that act as reductants and stabilizers for nanoparticles, as well as providing nanoparticles of a fixed size and stability (Gupta and Chauhan, 2017). The fungus *Fusarium solani* is one of the most common parasitic pathogens

that infect a large number of different crop plants in warm parts of the world, reaching more than 50 plant species belonging to different plant families, causing losses ranging between 30-80% (Haq et al., 2012). It is also one of the most dangerous causes of wheat seedling death and drop, root rot and wilting in tomato crops (Kipngeno et al., 2015).

So the aim of the research is to:

1. Biosynthesis of AgNPs from the fungus *Fusarium solani* using the green construction method and studying their properties.
2. Evaluating the efficiency of these particles in controlling seedling death and drop disease caused by the fungus *F. solani* on some Iraqi wheat varieties.
3. Testing the effectiveness of the reductase enzyme in producing AgNPs from the fungus *F. solani.*

Material and Methods

Preparing different concentrations of silver nitrate

Different concentrations of silver nitrate were prepared by adding AgNO3 silver nitrate powder to sterile distilled water in liter-sized beakers, as follows:

1. Prepare a concentration of 0.5 mM by dissolving 0.085 g/L of water
2. Prepare a 1 mM concentration by dissolving 0.17 g/L of water
3. Prepare a 1.5 mM concentration by dissolving 0.25 g/L of water
4. Prepare a 2 mM concentration by dissolving 0.34 g/L of water

After obtaining the required concentrations the beakers containing the different concentrations of silver nitrate were covered with aluminum foil and stored at a temperature of 4°C.

Solutions for the determination of nitrate reductase enzyme. The solutions were prepared according to the method Hassan and Hassan., (2021):

a) Preparation of solution (A): Prepare the solution from 30 mM AgNO3 with 25 ml propanol, 5% concentration, in a liter of phosphate buffer.

b) Preparation of solution (B): Prepare the solution from (58 mM sulphanilamide and 0.05 mM N-(1-naphthyl ethyiene diamine dihydrochloride (NEED)

Preparation of solid fungal medium: Potato Dextrose Agar (PDA)

Prepare the solid medium Potato Dextrose Agar (PDA) by dissolving 39 g of the prepared medium (Indian Media Hi Company) in one liter of distilled water according to the prepared company's instructions, then sterilize the medium in an autoclave at a temperature of 121°C and a pressure of 1.5 bar for 20 minutes afterwards. The medium was cooled to a temperature of 40°C, then the antibiotic Ampiclox was added at a rate of 100 mg/liter to the medium, and finally the medium was poured into Petri dishes and left to solidify at room temperature, and after solidifying, it was placed in the laboratory refrigerator.

Preparation of liquid fungal medium: Potato Sucrose Broth (PSB)

Prepare the liquid fungal medium, Potato Sucrose Broth (PSB), by adding 200 g of potato pieces to 2 liters of distilled water, taking into account the amount of water evaporated. Place the mixture in a 2-liter beaker on a heat source for 60 minutes. After the specified period of time has passed, it was Filter the mixture using filter paper to obtain the aroma. Complete the volume to one liter using distilled water Then 20 grams of sucrose were added to it, then it was stirred continuously for 30 seconds, after which the liquid fungal medium (PSB) was obtained. The medium was placed in 500 ml flasks, 250 ml/medium for each flask, then sterilized in an autoclave at a temperature of 121 °C and a pressure of 1.5 bar for 20 minutes later, the medium was cooled to a temperature of 30°C, then the antibiotic Ampiclox was added at a rate of 100 ml/liter of medium.

Inoculation of liquid medium (PSB) with the fungus *Fusarium solani*

The fungus *Fusarium solani* was inoculated by adding small pieces of the fungal colony, 1 cm in diameter, using a cork drill to the previously prepared liquid nutrient medium (PSB), The flasks were incubated in the incubator for seven days at a temperature 27 °C, taking care to move the flasks 3 times a day. Growth was monitored daily until a complete colony was obtained.

Preparation of silver nanoparticles from the fungus *Fusarium solani* according to the previously used method (Hassan and Hassan, 2019)

Preparation of silver nanoparticles from the filtrate, biomass and hot and cold extract of the fungus *Fusarium solani*

Filter the *F. solani* mushrooms growing in the liquid medium (PSB) using separate filter paper. After separating the biomass from the filtrate and obtaining the mushroom filtrate, the filtrate was placed in 10 ml tubes. Then the tubes were placed in the centrifuge for 10 minutes at a rate of 5000 rpm for the purpose of separation. The sediment from the liquid was collected in 5 ml and placed in 10 ml tubes. Then different concentrations of previously prepared silver nitrate (0.5, 1, 1.5, 2 mM) were added to it, each concentration separately in the form of drops, at the rate of 5 ml for each concentration, with the control treatment, which did not contain any of the concentrations mentioned, only the fungal filtrate clearing, which serves as Zero concentration, and finally the tubes were incubated according to the optimum temperature. For 72 hours in the dark, the tubes were followed for different time periods and the color change of the different concentrations was recorded.

Prepare the hot extract of the biomass of the fungus *Fusarium solani* by adding 1 gram of biomass/5 ml of distilled water at a temperature of 70°C. Place the mixture in a ceramic mortar. The mixture is crushed until reaching a state of homogeneity and obtaining the hot extract of the biomass. The extract was filtered using filter paper and transferred to 10 ml tubes. Then the tubes were placed in the centrifuge for 10 minutes at a rate of 5000 rpm for the purpose of separating the precipitate from the eluent. The eluent was collected in an amount of 5 ml and placed in 10 ml tubes, then different concentrations of silver nitrate (0.5, 1, 1.5, 2 mM) in the form of drops, 5 ml for each concentration, with the control treatment, which did not contain any of the concentrations, only biomass drippings from the hot extract, which is considered a zero concentration. The tubes were incubated in the dark for 72 hours, and the color variation for each concentration was recorded.

Prepare the cold extract of the cold biomass by adding 1 gram of biomass / 5 ml of distilled water at a temperature of 5 °C. Place the mixture in a ceramic mortar. The mixture is crushed until reaching a state of homogeneity and obtaining the cold extract of the biomass. Filter the extract using filter paper. Place the extract. In tubes with a capacity of 10 ml, the tubes were placed in the centrifuge for 10 minutes and at a rate of 5000 revolutions for the purpose of separating the sediment from the clear. The liquid was collected in a quantity of 5 ml, and the liquid was placed in 10 ml tubes, then different concentrations of silver nitrate (0.5, 1, 1.5, 2 mM), previously prepared in the form of drops, were added to it in the amount of 5 ml for each concentration, along with the control treatment that did not contain At any of the mentioned concentrations, only the biomass of the cold extract was detected, which is considered a zero concentration. The tubes were incubated in the dark for 72 hours for the purpose of obtaining color contrast.

Biomass was prepared by adding 1 gram of biomass/10 ml concentration of previously prepared silver nitrate (0.5, 1, 1.5, 2 mM), each concentration separately, except for the control treatment, which contained 1 gram of biomass/10 ml of distilled water. The tubes were incubated for 48 hours in the dark at room temperature, and after obtaining a color change, the mass was separated from the filtrate. The tubes were placed in the centrifuge for 10 minutes and at a rate of 5000 revolutions for the purpose of separating the precipitate from the eluent. The eluent was collected in an amount of 5 ml, then placed in 10 ml tubes and incubated at a temperature of 25 °C in the dark for 72 hours for the purpose of obtaining the color contrast.

Silver nanoparticle formation test

Chromatic contrast

Silver nanoparticles were detected by chromatography by changing their color state after 72 hours of incubation at a temperature of 25 °C, through changing the color of the filtrate from white to yellow and brown according to the concentration added to the fungal filtrate, the hot extract of the fungal biomass, and the cold extract of the fungal biomass and the mass. The vital activity of fungi individually (Al-Naimi, 2018).

Spectroscopic analysis using a spectrophotometer-uv-vis

The optical properties of silver nanoparticles resulting from mushroom filtrate, hot and cold extracts of biomass, and mushroom biomass were determined using a spectrophotometer. The sample was prepared by taking 2 ml of the previously prepared solutions. The samples were placed for examination with the above-mentioned device at a wavelength of 200-500 nanometers, and the values were recorded. Absorbance at each wavelength (Al-Shammari, 2015) .

Scanning electron microscope (SEM)

The structural characteristics of the sample were determined in terms of shape and size. The sample was prepared by placing drops of the previously prepared silver nanoparticle solution on a glass slide and drying it at a temperature of 60°C for 30 minutes. It was then dyed with crystal violet dye for 1 minute, then washed. With distilled water, the sample was fixed using a solution of Camza dye for 1 minute, after which the sample was plated with gold in a saturated gas surrounding. Arcon under a pressure of 50 Pascals and a current of 50 mA for 50 seconds. Then the sample was examined with a scanning electron microscope (SEM) at

75.000 X magnification. The experiment was used at the University of Technology at the Nanocenter according to the method used before (Elamawi et al., 2018).

Concentrations of silver nanoparticles prepared from the fungus *Fusarium solani* in the germination process of wheat seeds

Wheat seeds, variety (Sham 6), were treated with concentrations of silver nanoparticles from the fungus *Fusarium solani*, previously prepared for the fungal filtrate, and hot and cold extracts for biomass and fungal biomass at concentrations (0, 0.5, 1, 1.5, 2 mmM). The wheat seeds were soaked in the above-mentioned treatments. The tubes were left for 2 hours, after which the seeds were placed in petri dishes containing filter papers moistened with water. The plates were then incubated at a temperature of 25 °C, and the plates were followed after which the percentage of germination was recorded according to the following equation.

Germination rate % = Number of germinated seeds \ Total number of seeds × 100

Pathogenicity of the fungus *Fusarium solani*:

The pathogenicity of the fungus *Fusarium solani* was studied, estimated by the germination rate of wheat seeds, by placing a disk of a newly growing colony of the pathogenic fungus with a diameter (1 cm) in the middle of a Petri dish containing solid nutrient medium (PDA) using a cork drill. After the fungal colony grew and before it reached the edge of the dish, it was placed The wheat seeds were then incubated in the plates in the incubator at a temperature of 25 °C. Growth was monitored for different periods of time (4, 6, 8 days), and then pathogenicity was estimated based on the germination rates of the seeds (Al-Obaidi, 2012).

Estimating the effectiveness of the nitrate reductase enzyme

The effectiveness of the nitrate reductase enzyme was estimated following the method approved by (Hassan and Hassan, 2019), which is summed up by adding 2.5 ml of previously prepared concentrations of silver nanoparticles for each of (the fungal filtrate, the hot extract, the cold extract, and the fungal biomass, each separately, to 2.5 ml of solution (A) Previously prepared at pH 7.5, the mixture was incubated at 25 °C for 60 minutes, then 1.25 ml of solution (B) was added to it. To stop the reaction and note the color change to dark pink, the absorbance of the solution was measured at a wavelength of 540 nm using a

spectrophotometer, and the enzyme activity units were estimated based on the absorbance and according to the following equation:

$$\text{Enzymatic activity (units/ml)} = \frac{Absorbance\ at\ a\ wavelength\ of\ 540\ nm}{2.5 \times 60}$$

60 = reaction time (minutes)

2.5 = Added enzyme solution (ml)

Studying the effect of concentrations of silver nanoparticles on the fungus *Fusarium solani*, which causes seedling death and drop disease

The effect of concentrations of silver nanoparticles prepared in this study on the growth of the pathogenic fungus *Fusarium solani* was studied by placing a piece of the pathogenic fungus with a diameter of 1 cm in the middle of dishes containing solid nutrient medium (PDA) using a cork culturer, and upon the arrival of the fungal colonizer at a distance (1 cm) from the middle of the dish. The dishes were treated with different concentrations of silver nanoparticles (0.5, 1, 1.5, 2 mM). By making four holes at a distance of 2 cm from the fungal colony, concentrations of 0.1 ml were placed in the holes and incubated at a temperature of 25 °C. When the mushrooms had completed growing in the control treatment (distilled water instead of silver nanoparticles) to the end of the dish, the distance from the end of the dish was measured. Colony edge to pits containing silver nanoparticles (mm).

Statistical analysis

The research experiments were applied according to a completely randomized design (CRD) with a factorial experiment, and the results were analyzed using the Statistical Analysis System -SAS (2012). The averages were compared according to the least significant difference (LSD) test under the 0.05 level (Al-Rawi and Abdel Aziz, 2000).

Results and Discussion

Detection of silver nanoparticles by contrast chromatography

The results are shown in Figure (1) to detect the biosynthesis of silver nanoparticles using color variation to change the color of the mushroom filtrate, the mushroom biomass, and the hot and cold extract of the fungus *Fusarium solani* biomass from colorless to yellow and brown with increasing concentration after 72 hours of adding silver nitrate. To mushroom filtrate, mushroom biomass, and hot and cold extracts of *Fusarium solani* fungal biomass,

This is evidence of confirmation of the synthesis of ultra-small silver nanoparticles by color change within the solution, while no color change appeared in the control solution.

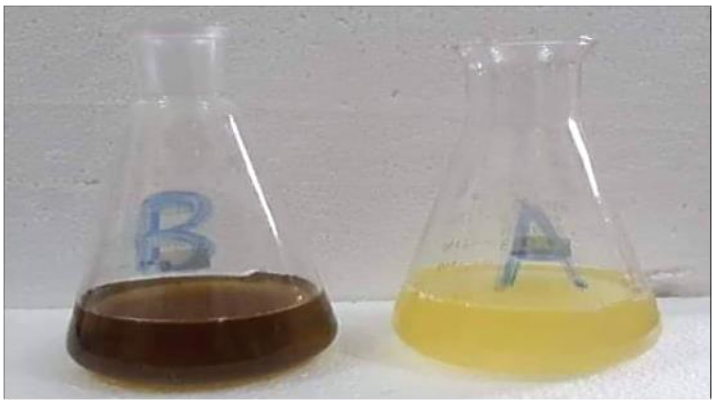

Figure 1. Color variation of *Fusarium solani* fungus filtrate 72 hours after adding silver nitrate

The explanation for this phenomenon is attributed to the phenomenon of surface plasmon resonance. This phenomenon has a property that occurs in many metals, including silver, as a result of the diameter of its particles reaching the nanometer scale. Therefore, the Spectrophotometer UV.VIS spectrophotometer is used at different wavelengths to prove the formation of Silver nanoparticles (Krishnaraj et al., 2010; Bamsoud and Bahwirth, 2017). This result was consistent with (Mahmoud, 2017) When he noticed a color change in the filtrate of the fungus Entomophthora muscae after adding a solution of silver nitrate after 72 hours, from colorless to yellow-brown, which confirmed the formation of silver particles at wavelength 420 using a spectrophotometer.

The optical spectrum of the studied fungi

Optical spectrum of cold *Fusarium solani* extract

The results of the optical spectrum in Figure (2) for the cold extract of the *Fusarium solani* show that there is an increase in the absorbance values with increasing concentration of the cold mushroom extract. The results also show that the highest range of absorption was within the wavelength 400_450, as the highest absorbance value reached 0.71 at the wavelength 450 for concentration 2 mM, followed by 0.66, 0.61, and 0.5 for concentrations of 1.5, 1, and 0.5 mM, respectively, at the same wavelength.

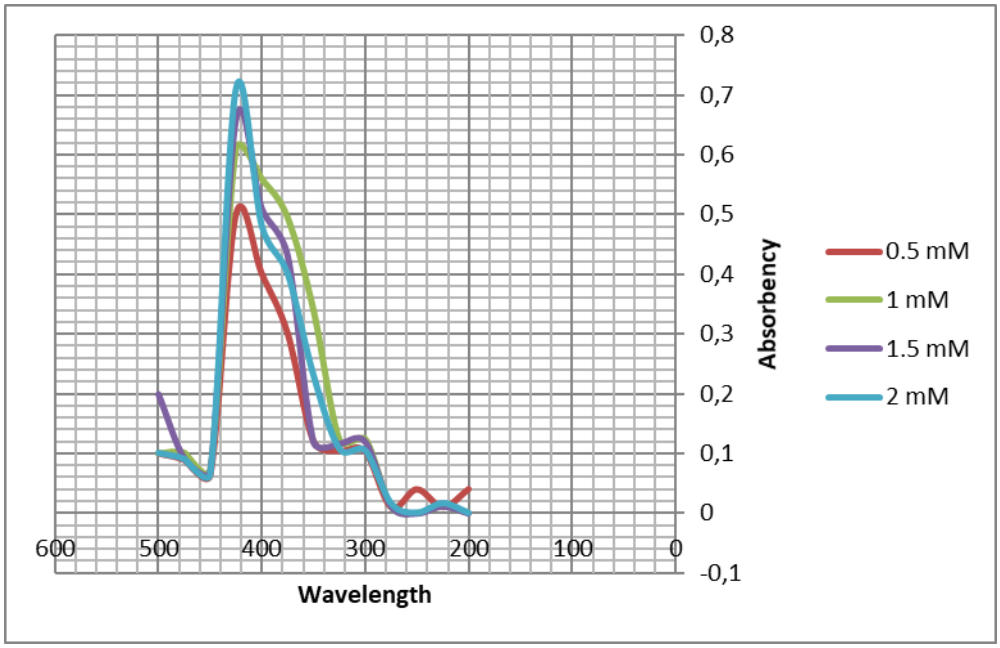

Figure 2 of the cold extract of the fungus *Fusarium solani*

Optical spectrum of *Fusarium solani* extract:

The results in Figure (3) show that there is an increase in absorbance values with increasing concentration of the hot *Fusarium solani* extract, that is, there is a direct relationship between absorbance and concentration, that is, the higher the absorbance, the higher the concentration. It is noted from the results in this figure that the highest range of absorption was within the wavelength. 375_425, as the highest absorbance value was 0.81 at wavelength 400 for the concentration of 2 mM, followed by concentrations of 1.5, 1, and 0.5 mM, as the absorbance reached 0.73, 0.63, and 0.55, respectively, at the same wavelength.

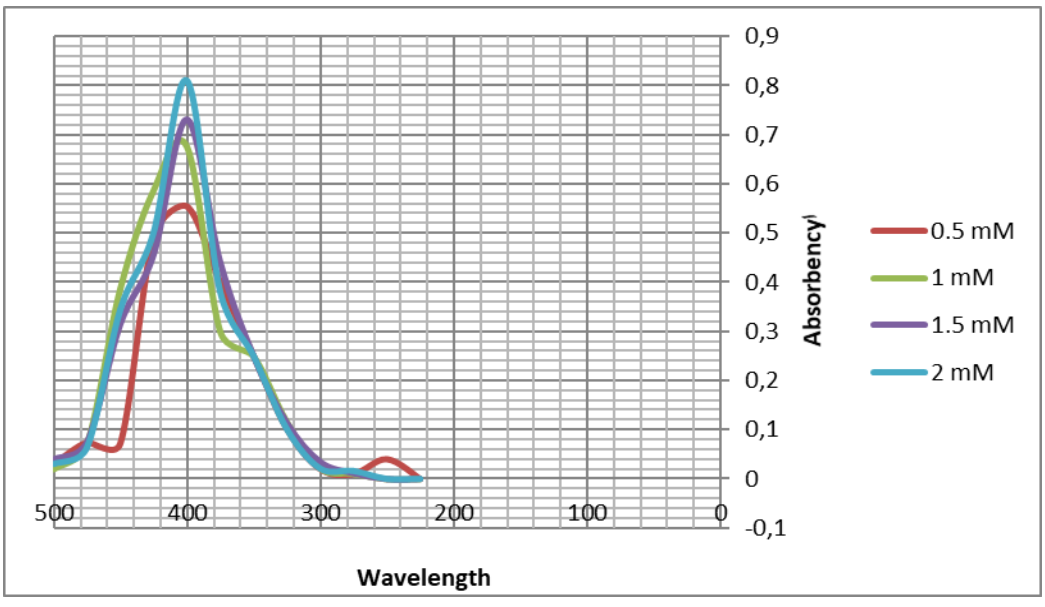

Figure 3. Optical spectrum of hot extract *Fusarium solani*

Optical spectrum of Fusarium solani fungus filtrate

The results of the spectrophotometric analysis show that the highest absorption range was within the wavelength 400-450, as the highest absorption value reached 1.78 for the concentration of 2 mM at the wavelength of 425, followed by 1.66, 1.61, and 1.55 for the concentrations of 1.5, 1, and 0.5 mM, respectively, at the same wavelength of filtrate. *Fusarium solani* As the results in Figure (4) show, there is an increase in absorbance values with increasing concentration of the fungal filtrate at all wavelengths.

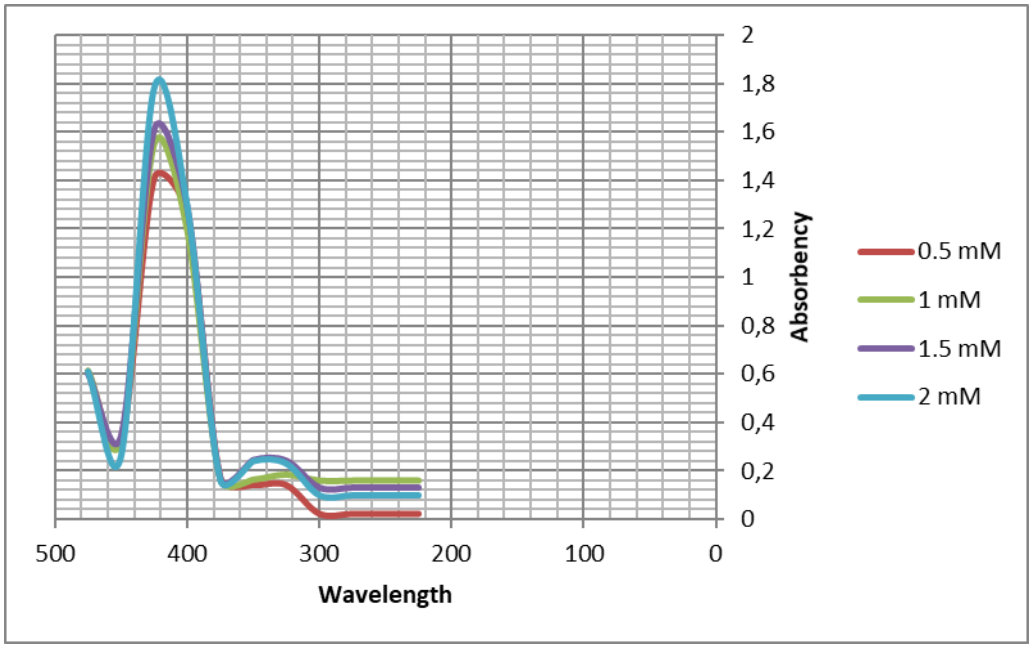

Figure 4. Optical spectrum of the fungus *Fusarium solani* filtrate

Optical spectrum of biomass of the fungus *Fusarium solani*

The results are shown in Figure (5). When the concentration increases, the absorbance values increase at all wavelengths of the *Fusarium solani* fungus biomass extract. The results of the optical spectroscopic analysis of the fungus biomass extract show that the highest range of absorption was within the 400-450 wavelength, as the highest absorbance value reached 2.037 at the wavelength. 425 for the concentration of 2 mM, followed by 1.96, 1.89, and 1.77 for the concentrations of 1.5, 1, and 0.5 mM, respectively, at wavelength 425.

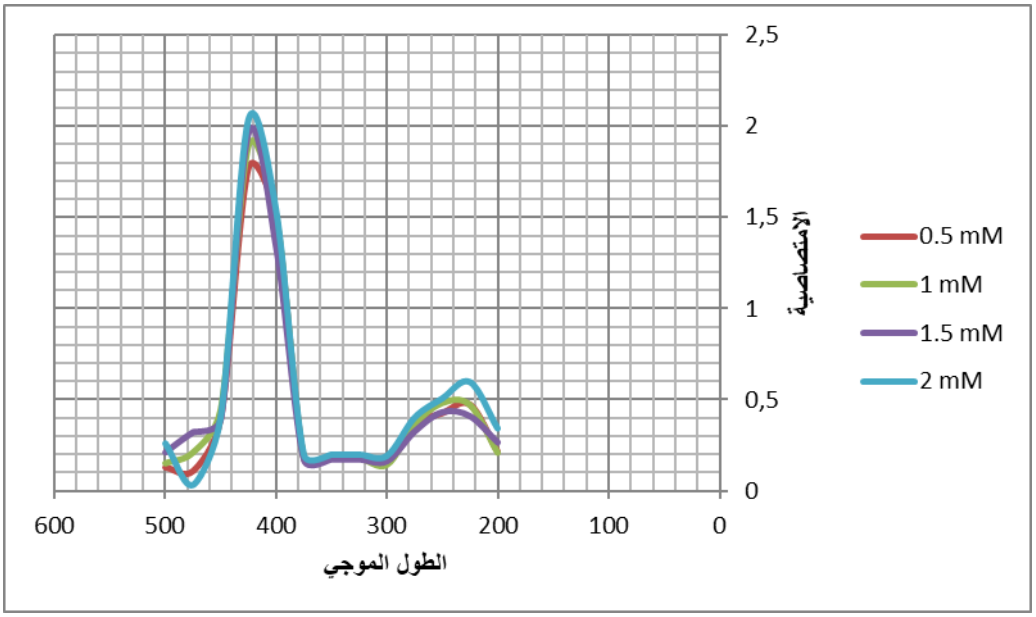

Figure 5. Optical spectrum of the biomass extract of the fungus *Fusarium solani*

The results above explain that the most important reason for the increase in absorbance with increasing concentration may be attributed to the fact that these fungi have a high effectiveness in the synthesis of silver nanoparticles, and according to the concentrations studied, the higher the concentration, the greater the result of an increase in the synthesis of Ag nanoparticles and thus an increase in the silver particles formed, as the higher the concentration, the greater the The value of absorbance and the intensity of absorbed light, i.e. there is a direct relationship between them (Bhattacharyya et al., 2012). The most important range of wavelengths for silver particles falls within (390-475), which achieves nanoparticle sizes within 5_100 nanometers manufactured by fungi (Soni and Prakash, 2013). The results of previous studies indicated that the nanoscale dimensions of (5- 100) and according to the wavelengths of the nanoparticles manufactured by fungi for (cold extract, biomass, and filtrate), which were within the nanoscale range of (10 - 100), and this is what achieves the formation of silver nanoparticles in The three cases. These results were consistent with (Bhainsa and D'Souza, 2006) after they obtained the highest absorbance at a wavelength of 420 nm from the fungus Aspergillus fumigatus, which indicates the formation of silver nanoparticles in the solution of the leachate, biomass, and cold extract. These results also agreed with Vahabi et al., (2011) when silver particles were formed from the fungus *Trichoderma* sp at wavelength 420.

As for the hot extract, it was smaller in size than the nanoscale (10-100) in some cases due to the formation of silver nanoparticles. The reason for this is due to the lack of reduction of silver nitrate by the nitrate reeducates enzyme that is released by in the solution of the hot

mass extract. Which has the ability to reduce metals, especially silver nitrate (Al-Shammari, 2015). The reason for this is also due to the lack of irritation of plasmon vibrations at the metal surface, which led to the non-formation of silver nanoparticles of their normal sizes (Adebayo and Oloke, 2017).

Scanning electron microscope (TEM)

The results in Figure (6) show the detection of silver nanoparticles by scanning electron microscopy (TEM), the formation and determination of the shape and size of silver nanoparticles in oval and spherical shapes, and the size of silver nanoparticles at nanosizes of 30-70 nm.

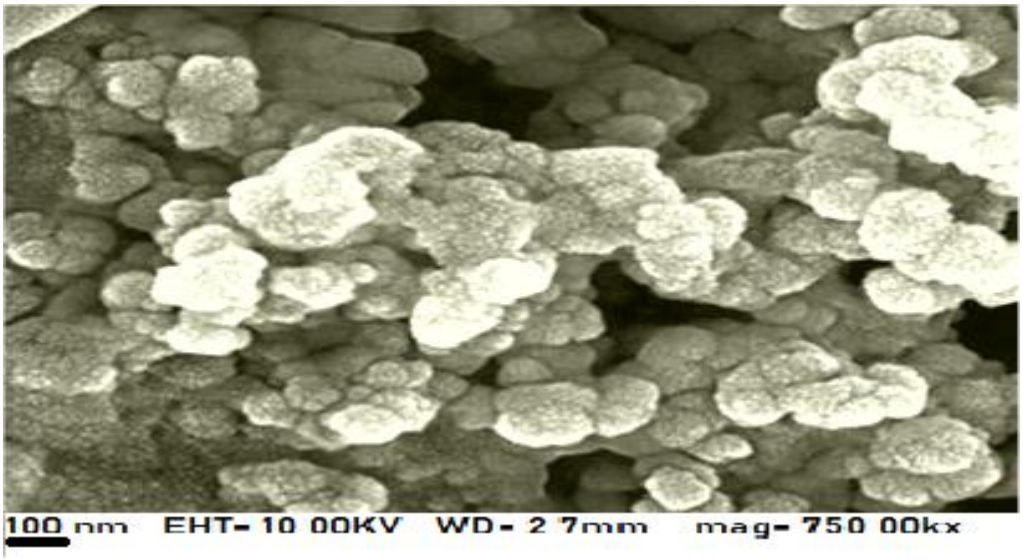

Figure 6. Scanning electron microscope of silver nanoparticles (AgNPs) at 75,00kx magnification

The above results prove the formation of silver nanoparticles at sizes 30-70 nanometers, and this is what was stated in the study (Gopinath and Velusamy, 2013), which confirms that nanoparticle sizes fall between 5-100 nanometers, and these results are consistent with (Duran et al., 2005) and also agreed with (Al-Naimi, 2018).

Effect of concentrations of silver nanoparticles prepared from the fungus *Fusarium solani* on germinating seeds of wheat variety (Sham 6) %

The results in Table (1) indicate the effect of concentrations of silver nanoparticles prepared from Fusarium solani on the percentage of germination of seeds of wheat variety (Sham 6), as the fungal biomass treatment outperformed the rest of the treatments, reaching 97.01%, with no significant differences with the hot extract treatment of the mass. The vitality

of the mushrooms reached 97.00% compared to the treatments of cold extract of mushroom biomass and mushroom filtrate, which reached 96.85% and 96.71%, respectively.

With regard to concentrations, the 0.5 mM concentration showed the highest seed germination rate, reaching 97.54%, with no significant differences with the 1 mM concentration, which reached 97.33%, compared to the 1.5 and 2 mM concentrations, which gave 96.85 and 96.78%. As for the interaction The treatment with mushroom filtrate at a concentration of 0.5 mM showed the highest seed germination rate, reaching 98.08%, with significant differences with the treatment consisting of cold extract of biomass, which showed the lowest seed germination rate, giving 95.10%.

Table 1. Effect of concentrations of silver nanoparticles prepared from the fungus *Fusarium solani* on the germination process of wheat seeds (Sham 6) %

Transactions of silver nanoparticles	**Concentrations mM**					**Transaction rate**
	0	**0.5**	**1**	**1.5**	**2**	
The filtrate	96.36	98.08	97.95	95.84	95.34	96.71
Biomass	96.74	97.61	97.66	96.24	96.83	97.01
Cold extract	95.10	96.88	96.82	97.69	97.79	96.85
Hot extract	95.67	97.60	96.96	97.63	97.17	97.00
Concentration rate	95.96	97.54	97.33	96.85	96.78	96.89

Minimum significant difference L.S.D. $_{0.05}$ for treatments 0.39 for concentrations 0.44 Interaction 0.95

The interpretation of the above results is that the concentrations of silver nanoparticles did not have any negative effect on the germination process of wheat seeds in all fungal treatments of silver nanoparticles, and that silver nanoparticles have the ability to increase the water content, which in turn leads to an increase in moisture content. Moreover, Silver nanoparticles increase cell division, which leads to a higher germination rate in plants (Farooq et al., 2005). The reasons for the decrease or increase in germination % may also be attributed to other compounds present in the fungal filtrate, biomass, or both hot and cold extracts, and such compounds (alkaloids, phenols, enzymes). The presence of such compounds or their interference with the concentrations of silver nanoparticles may encourage or discourage germination. Seed germination (Wuttipong et al., 2017). On the other hand, the studied fungi are different species and therefore there is a difference in their genetic structure and physiological behavior, which is reflected in the production of medicinal compounds that may encourage or inhibit seed germination (Chang and Miles, 2004).

The pathogenicity of the fungus *Fusarium solani*, which causes death and fall of wheat seedlings, estimated by the percentage of germination of wheat seeds, Sham 6 variety

The results in Figure (6) show the pathogenicity of the pathogenic fungus *Fusarium solani* on the germination rate of wheat seeds (Sham variety 6), as it is noted that the pathogenic fungus has a high impact on the germination rate starting after four days of incubation, as the germination rate of wheat seeds reached 52.67%. After six days of incubation, the germination rate decreased due to the influence of the pathogenic fungus, reaching 26.6%. After eight days, the effect of the pathogenic fungus was very high, as the germination percentage decreased to 11.48%.

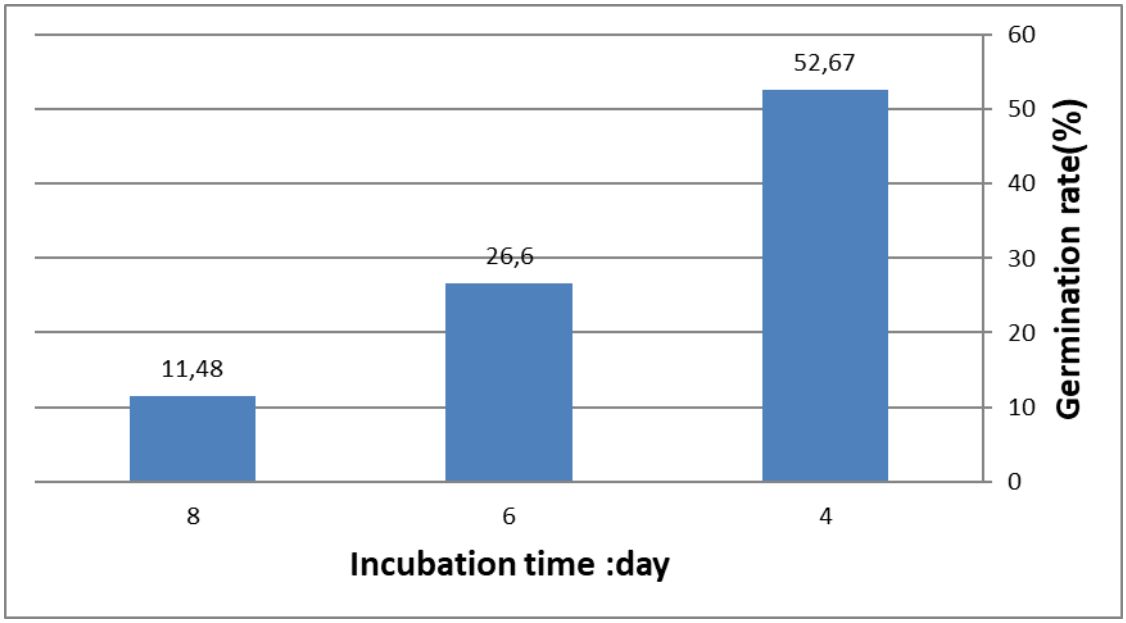

Figure 6. Pathogenicity of the fungus *Fusarium solani* estimated by the percentage of germination of wheat seeds, Sham 6 variety. (LSD $_{0.05}$: 9.15)

The reason for this is that the pathogenic fungus *Fusarium solani* has high virulence in attacking its plant hosts. The fungus showed high virulence towards seeds through direct penetration due to mechanical pressure by the appressorium, which in turn works to penetrate the cells of the epidermis and cuticle through mechanical penetration as a result of the pressure of the fungus on the epidermis and also Through the production of cell wall-degrading enzymes, it kills the tissue by producing enzymes and hydrolytic toxins that lead to dissolution, including Lytic enzymes, which work to completely destroy and collapse the cell walls of the infected grains in the penetration area to help the fungus spores penetrate (Schroeder et al., 2013).

Estimation of the activity of the nitrate reductase enzyme using concentrations of silver nanoparticles prepared from the fungus *Fusarium solani*

The results of the effectiveness of the nitrate reductase enzyme using concentrations of silver nanoparticles prepared from the fungus *Fusarium solani* in Table No. (2) show that filtrate treatment was superior to the other treatments. The results also show that the 1.5 mM concentration in the filtrate treatment was superior to the rest of the concentrations of the treatments, as it showed the highest rate of enzymatic activity, reaching 0.63 units/ml, followed by the same concentration from the same treatment, reaching 0.52 units/ml, compared to the 0 mM concentration of Treatment of the hot extract of biomass reached 0.02 units/ml. The results also showed an increase in the activity of the enzyme for all treatments by increasing the concentration up to a concentration of 1.5 mM, then the activity decreased at a concentration of 2 mM.

Table 2. Estimation of the effectiveness of the nitrate reductase enzyme using concentrations of silver nanoparticles prepared from the fungus *Fusarium solani*

Transactions of silver nanoparticles	**Concentrations mM**					**Transaction rate**
	0	**0.5**	**1**	**1.5**	**2**	
The filtrate	0.055	0.42	0.49	0.63	0.47	0.41
Biomass	0.027	0.24	0.31	0.37	0.34	0.25
Cold extract	0.032	0.36	0.43	0.52	0.38	0.34
Hot extract	0.022	0.15	0.21	0.26	0.18	0.16
Concentration rate	0.033	0.29	0.36	0.44	0.34	0.29

Minimum significant difference $L.S.D._{0.05}$ for treatments 0.017 for concentrations 0.011 Treatments x concentrations 0.21

The above results explain that the enzyme nitrate reductase, released by microorganisms, is one of the most important factors in the synthesis of silver nanoparticles. Studies have shown that NADH- and NADH-dependent enzymes, especially nitrate reductase, are important factors in the biosynthesis of metal nanoparticles (Hassan and Hassan, 2021). During the reduction process, nitrate is converted to nitrite which in turn transfers electron to silver ions and as a result, silver ions are converted into silver nanoparticles (Ghorbani, 2012) The reason for the superiority of the filtrate as having the highest enzyme activity is that the fungus produces the enzyme in its extracellular form to a greater extent than from the inside of the cell. As for the cold extract of the biomass, the cooling conditions preserved the effectiveness of the enzyme, which led to the enzymatic activity of the cold extract being higher than that of the hot extract, which in turn was affected by the heat because the enzyme

consists of a protein and a mineral part. The reason for the decrease in the enzymatic activity of the biomass is that the biomass Limited enzyme production for biomass in the presence of different concentrations of silver nitrate compared to the filtrate, in which the enzyme is produced by the fungus throughout the incubation period (Anil et al., 2007).

The reason for the decrease in enzymatic activity at a concentration of 2 mM is due to the presence of compounds within the filtrate, the hot and cold extract, and the biomass of the fungus, which have the effect of interfering with high concentrations of silver nanoparticles, which led to a decrease in the effectiveness of the enzyme and such compounds (alkaloids, phenols, enzymes, and (products fungal), The materials resulting from enzymatic activity are determined by two factors: the first is the enzyme and the second is the concentration of the base material with some reaction conditions. Whenever the silver ions increase to the highest limit of the amount of the enzyme in the samples, this means that the enzyme is unable to convert higher concentrations, and with changing reaction conditions (time + pH) it affects This negatively affects the work of the enzyme as well, and this leads to a decrease in the enzyme activity at a concentration of 2 mM, and the reason for the decrease in concentration is 0 mM. Due to the absence of the base material for the enzyme, the reason for the maximum enzymatic activity at the concentration of 1.5 mM is due to the high enzymatic activity of the nitrate reductase enzyme, which converts silver ions into silver nanoparticles (Hamedia et al., 2017).

Effect of concentrations of silver nanoparticles prepared from filtrate, mass, hot and cold extracts of Fusarium solani on inhibition of growth of the pathogenic fungus Fusarium solani

The results are shown in Figure (3) of the effect of concentrations of silver nanoparticles prepared from filtrate, mass, and hot and cold extracts of the fungus *Fusarium solani* in inhibiting the growth of the pathogenic fungus Fusarium solani, until there is an increase in the rate of inhibition when the concentration increases up to a concentration of 1.5 mM in all treatments, as the results show. To the superiority of all treatments in inhibiting the pathogenic fungus *Fusarium solani* over the treatment of hot extract of the fungus biomass The results also show that the mushroom filtrate recorded the highest rate of inhibition at the concentration of 1.5 mM, reaching 0.64 mM, compared to the hot extract treatment at the concentration of 0.5 mM, reaching 0.11 mM.

Table 3. Effect of concentrations of silver nanoparticles prepared from filtrate, bulk, and hot and cold extract of *Fusarium solani* fungus

Transactions of silver nanoparticles	Concentrations mM					Rate
	0	**0.5**	**1**	**1.5**	**2**	
The filtrate	0	0.14	0.26	0.64	0.48	0.30
Biomass	0	0.16	0.23	0.41	0.25	0.21
Cold extract	0	0.18	0.26	0.55	0.43	0.28
Hot extract	0	0.11	0.16	0.17	0.13	0.11
Concentration rate	0	0.15	0.23	0.44	0.32	0.23
Minimum significant difference L.S.D. $_{0.05}$ for treatments 0.053 for concentrations 0.059 Treatments x concentrations 0.11						

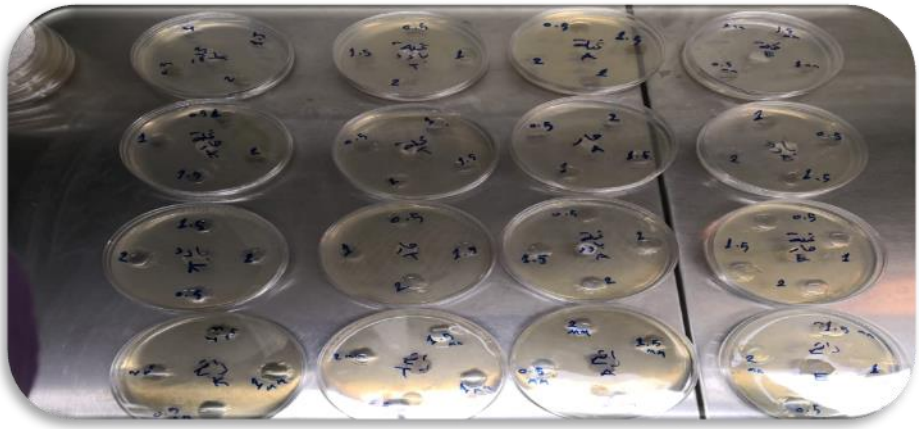

Figure 7. Experiment on the effect of concentrations and treatments of silver nanoparticles on inhibiting the pathogenic fungus *Fusarium solani*

The reason for the inhibition of fungi by concentrations of silver nanoparticles is that silver nanoparticles have the ability to affect the DNA of the pathogenic fungus by causing the DNA to lose its ability to copy and replicate, which leads to a defect in the process of cloning the DNA strand (Kim et al., 2012). It also has the ability To penetrate the cell walls (Al-Wakeel, 2103). This is because silver nanoparticles have the ability to adhere to the walls of fungal cells and then penetrate, analyze and absorb the cell walls through interaction with the metabolic and biological processes within the fungus, which leads to influencing the regulatory processes of the proteins and enzymes of the fungus and thus inhibiting the fungus (Narayanan and Hyun, 2011). They also have an effective role in damaging the proteins, fats, and nucleic acids of fungal cells. Nanoparticles can also bind directly to the fungal cell membrane, leading to the destruction of spores, fungal hyphae, and reproductive structures of the pathogenic fungus (Hwang et al., 2008).

Conclusions

1. The high ability of the pathogenic fungus *Fusarium solani* to produce silver nanoparticles.
2. Silver nanoparticles biosynthesized from the pathogenic fungus *Fusarium solani* have no effect on the germination rate of wheat seeds.
3. High efficiency of the reductase enzyme in the process of synthesis of silver nanoparticles.

Recommendation:

1. The use of biosynthetic silver nanoparticles from the fungus *Fusarium solani* to inhibit plant fungal pathogens.
2. Use of silver nitrate in the synthesis of nanoparticles
3. Using fungi in the biosynthesis of nanoparticles because of their high ability in the biosynthesis process.
4. Testing the effectiveness of the reductase enzyme in using the biosynthesis process of nanoparticles for the compatibility process between the particle production process.

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