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## Applications of silver nanoparticles against stored grain (wheat seed) mycoflora

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### summary:

The current study included the isolation and identification of fungi associated with the seed of wheat plant, which were considered important agricultural crops globally, where the isolated fungi were diagnosed by traditional methods as well as by PCR. Genetic sequencing and genetic tree were also conducted to monitor the evolutionary relationship of some fungal isolates under study. The study also included the preparation of different concentrations of silver nanometers for the purpose of testing their effect on isolated fungi in invitro (laboratory) conditions as well as in the soil in vivo.

The results of our current study showed the superiority of superficially sterilized seeds over non-superficially sterilized seeds in terms of the number and frequency of isolated fungi, which included *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus oryzae*, *Rhizopus oryzae*, *Alternaria alternata*, and *Alternaria solani*.

In wheat seeds the fungus *A.niger* It was the most frequent in both sterile and non-superficially sterilized seeds where it was 45.2% and 25.6%, respectively.

The genetic study via PCR, gene sequencing and genetic tree showed that the isolates were identical to the traditional screening that relied on phenotypic traits, and the molecular biological study also showed that our isolates were close to isolates and Pakistani (*A.niger*), Indian (*A.terreus* and *A. solani*), Chinese (*A.fumigatus* and *R. oryzae*) and American (*A.parasiticus*).

With regard to the effect of silver nanoparticle in plates (well diffusion method), the current study showed the existence of different effects of it, as the treatment of all concentrations (5, 10 and 15) % did not differ significantly, but it gave an inhibition that reached above in the fungus *A.niger* at a concentration of 15% for silver nanoparticles, where it reached 11.33 mm, although these particles gave a clear inhibition, but they remained less than what the fungicide Difenostar (positive control), which outperformed nanoparticles in all parameters.

The effect of treatment with nanoparticles was varying on the germination rate of wheat and barley seeds in the plates, the seeds gave a germination rate of 100% when using silver nanoparticles for wheat, while the rest of the concentrations gave a germination rate of 100% for wheat. As for the root and the peduncle, they were affected by the treatment of nanoparticles above, and the most affected treatment was the treatment of 5% of silver nano, where the length of the stem was 30 mm.

In field experiments, all the treatments of the seeds gave the dusty and non-dusty seeds a single germination rate, which is 100%, and the nanoparticle coefficients were significantly influential, as they caused an increase in the length of the root and the peduncle, , as well as the concentration of 5% Nano silver on the peduncle and 10% on the wheat root.

**Key words:** Fungi, Molecular identification, Nanoparticles, Pesticides, Iraq.

## Introduction:

Wheat is among the most important crops cultivated in the world, among the most widespread and consumed crops, whether for human or animal nutrition. Increasing agricultural production is a means to ensure food security for people, especially for developing countries, as production, especially in the field of grain, is fluctuating and does not meet the needs of a number of citizens. The population is continuously increasing as a result of several factors that impede production, including climatic diseases, soils, and crops (Pumlet, 2021). The fungi accompanying seeds are one of the most common problems that farmers suffer from, as they affect the seeds in terms of quantity and quality, as they lose them to the required size, shape and weight, not to mention that they become undesirable in the market, which constitutes a real problem with multiple aspects (Ziani *et al.*, 2009).

The problem becomes more complicated when the target crop is an important strategic crop for food security, such as wheat and barley, which are the two main crops on which the lives of billions of people depend, as well as animals, especially livestock (Line, 2002). Agricultural crops are susceptible to infection with two types of fungi: field fungi and storage fungi. They include several

genera, the most important of which are *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Alternaria*, *Rhizopus*. (Belkacem-Hanfim *et al*, 2013) and in general, the grains are infected with many fungi, which are divided into two main groups:

1. Field fungi (which affect crops within the field)
2. Storage fungi (which affect grain after harvest and during storage), to which many genera belong, such as *Aspergillus*, *Alternaria*, and others.
3. Numerous researches have shown that silver nanoparticles are among the most commercially used nanomaterials for plant growth and disease resistance , where many researchers have tried to understand the effect of silver nanoparticles on germination and growth of many crops, as (Abu Zaid and Mustafa, 2014) studied the effect of silver nanoparticles on two types of wheat and barley, and noticed an increase in the percentage of germination, stem length and root length decrease compared to the results of comparative laboratories (control) (Hajjat ,2015) indicated that low concentrations of silver nanoparticles led to an increase in seed germination, seedling growth, and the length of the roots of the fenugreek plant. (Yin *et al* ,2012) investigated the effect of silver nanoparticles on the germination of eleven types of wild plants, and the results of this study found that silver nanoparticles enhanced the germination rate of one species.

### **Research objectives:**

This study aimed to

1. To isolate, identify and purify fungi from wheat seeds stored in silos and commercial stores and prepare different dilutions of silver nanoparticles and to treat fungi by wells method and to find out the best concentrations for treatment.
2. To treat the seeds by covering them with these particles and then testing their effect on the seeds in terms of germination, vitality, root length, feather, root and leaf set.

### **The importance, value and application of the research's results**

The study included the use of silver nanoparticles to combat the pathogenic fungi associated with wheat seeds to reduce the use of pesticides that have been proven to us to cause unwanted side effects. Its importance in reducing pollution resulting from the use of chemical pesticides, reducing side

effects, material cost, the effort exerted by farmers and obtaining high quality healthy seeds and a large germination rate.

## **Materials and Methods**

### **Collection of samples:**

Seed samples of wheat under study was collected from the General Company for Grains / Silo Diwaniyah the stores of the Mesopotamian Company for Seed Production / the warehouse complex (Al-Banaker) The General Company for Grain Trade / Silo Sumer in Diwaniyah Governorate, randomly in the month of June on 14/1/2022, at the rate of (1 kg) for each sample. The samples were placed in dark paper bags and labeled. And brought to the laboratory. This study was conducted in the laboratory of (Diwaniyah Directorate of Agriculture) / Diwaniyah Governorate.

### **Isolation and Identification of fungi**

The fungal species isolated from samples of wheat grains growing on PDA medium were diagnosed based on the phenotypic characteristics of the colonies, where the general characteristics of each isolate were recorded in terms of colony shape, color, texture, diameter, edges and height of the colony in addition to the pigments it produces, as well as relying on the microscopic characteristics through following methods The following diagnoses:

Microscopic examination according to what was stated in Moris et al. (2008) and depending on the sources mentioned by each of Ellis, (1994); Quinn, *et al.* (2002); Houbraken *et al.* (2008) and Webster and Neber (2007).

### **Testing the effect of silver nanoparticles on inhibition of antifungal activity of silver**

#### **Well diffusion method**

The fungal samples were planted in sucrose potato broth and incubated for 5-7 days at a temperature of 25 °C, after which a volume of 5 ml of the fungal culture was mixed with cooled dextrose potato agar after sterilization. ml using a corkscrew with a diameter of 5 ml and at the rate of 5 holes in one dish, 100 microliters of each concentration of nano-solutions (10,5,15%) were transferred. In addition to transferring 100 microliters of each of distilled water and Difenostar pesticide (which was prepared according to the recommended instructions on the package, in three replicates, the dishes were incubated for 7 days at a temperature of  $\pm 25$  °C, after which the inhibition area that appeared

around the pits was measured in mm by a measuring ruler Two perpendicular diagonals. (Balouiri *et al.*, 2016).

### **Testing the effect of nanoparticles in the laboratory on the vegetative and root systems of the plants under study**

Wheat grains were prepared. The grains were washed with water well, then placed in sodium hypochlorite solution (NaOCl at a concentration of 2%). The immersion process took 3 minutes with continuous stirring to get rid of any contamination in the grains. After that, the grains were washed with water again several times to get rid of the residues of the solution. the hypochlorite, and thus the grains are ready to be used in the experiment. The grains were immersed in concentrations of nanoparticles of nano-silver oxide (5%, 10%, 15%) prepared previously and Difenostar) and distilled water for (20) minutes (Balouiri *et al.*, 2016).

The culture process was carried out by placing 10 grains in each Petri dish containing filter paper with three replicates, then 5 ml of water was added to each dish every three days and the dishes were placed in the incubator temperature (at a temperature of  $25 \pm C$ ), then the readings were taken after a week. The following measurements were taken:

#### **A. Germination percentage:**

It was calculated using the following equation:

Germination percentage (%) = (number of germinated grains / total number of grains) x (100). (Bamomen 1994)

#### **B. Feather length (cm)**

The reading was taken from the junction of the stem with the root to the highest level.

#### **C. Length of the root (cm)**

The readings were taken from the root-to-stem junction area below level.

#### **D. Fresh and dry weight of feather and root, gm:**

The shoot and root were weighed separately using a sensitive electronic balance, where the fresh weight of each of the feather and root was taken for all treatments, and the feather and root were dried using an incubator at a temperature of 60 C until the weight stabilized.

A graduated ruler with high transparency was used to measure the length of the shoot and root, and the average lengths for each dish were calculated by dividing the total lengths by the number of plants.

### **The field parts**

#### **The effect of nanoparticles on the vegetative and root systems of the plants under study**

To investigate the effect of the nanoparticles under study (nano silver) on the germination of wheat seeds in the field, three concentrations of nanocomposites (10, 5, 15%) were prepared, and the fungicide treatment (Difenostar) was used, and according to the recommended instructions, after that the seeds were treated the soil was prepared by bringing it from one of the wheat fields in the city of Diwaniyah and left without sterilization. Pots with a diameter of 11 cm and a height of 10 cm were filled with soil in equal quantities, after which the wheat seeds were sown. treatment, with 3 seeds for each rootstock, and with three replicates for each treatment, and the cultivation was at the appropriate time for the cultivation of wheat in the field (Sarhan *et al*, 2001).

The readings were taken 14 days after planting the seeds with continuous follow-up and according to what was stated in the above paragraph regarding the measurement of germination percentage, stem length, root and fresh and dry weight in the different treatments.

### **Statistical analysis**

The data of the results of the study were analyzed statistically using the statistical program Statistical Package of Social Science (SPSS) at a probability level of  $P \leq 0.05$ .

### **Results and discussion**

#### **Isolation & identification of fungi**

Several types of fungi associated with wheat seeds were isolated and identified from the General Grain Company/Diwaniyah Silo and the stores of the Mesopotamia Seed Production Company. and the General Company for Grain Trade / the warehouse complex (Al-Banaker) and the General Company for Grain Trade / Silo Sumer in Diwaniyah Governorate. Which is *Aspergillus niger*, *Aspergillus terrus*, *Aspergillus parsiticus*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus oryzae*, *Rhizopus oryzae*, *Alternaria alternata*, *Alternaria solani*

The results in table (1) showed a variation in the relative percentage frequency of the fungi diagnosed in wheat seeds that is not surface sterilized and is surface sterilized. It was noted that the relative percentage frequency of the seeds that is surface sterilized is higher than that of the seeds that is not surface sterilized. This is because sodium hypochlorite is sterile, but its effect is mainly limited to the surface fungi carried on the grain that is contaminated with fungi. Washing and sterilization affects the fungi that is carried on the grain to reduce the presence of (Sirhan *et al.* 2001), who emphasized the effect of sodium hypochlorite on externally carried fungi on seeds. The results also showed that *Aspergillus niger* was highest in treatment of unsterilized and sterilized wheat seeds (Table 1), with relative frequency of 45.2% and 25.6%, respectively, compared to *Rhizopus oryzae*, which was isolated from seeds, which had highest frequencies of 55.3% and 17%, respectively. These findings are consistent with (Sarhan *et al.* 2001), This may be due to the high production capacity of the reproductive units of *Aspergillus* and *Rhizopus*, giving them the opportunity to grow and appear (Jawtez *et al.* 1998) These findings are consistent with (Diener *et al.* 1987) finding that various species of *Aspergillus* are often seed companions due to the high prevalence of this group of fungi. *Aspergillus* and several other fungi attack grains in the field by invasive fetus and other parts of the seed. Reddy, 1992 stated that the long storage time of the seeds with the relative humidity and temperature appropriate for the growth of the fungi, as well as the presence of insects and rodents, are important factors responsible for increasing the incidence of store fungi. *Rhizopus* frequency decreased in the treatment of surface-sterilized wheat seeds to 8.1% for surface-sterilized seeds, 48.4% for surface-sterilized seeds, 17% for surface-sterilized seeds, and 55.3% for surface-sterilized seeds for barley, due to the fact that *Rhizopus* is a fungus that is carried on the exterior of the seeds and is not associated with the inside of the seeds and does not infect a fetus (Saadoun, 2005).

**Table (1) Fungi isolated from non-surface sterilized and sterilized wheat seeds and their frequency ratios**

T	Isolated fungi	Superficially unsterilized seeds	Percentage	Surface sterilized seeds	percentage
1	<i>Aspergillus niger</i>	14	37.8	19	28
2	<i>Aspergillus terrus</i>	-	-	7	10
3	<i>Aspergillus parasiticus</i>	2	5.4	11	16

4	<i>Aspergillus fumigatus</i>	-	-	4	6.5
5	<i>Aspergillus flavus</i>	-	-	5	7
6	<i>Aspergillus oryzae</i>	-	-	4	6.5
7	<i>Aspergillus ochraceus</i>	-	-	2	3
8	<i>Rhizopus oryzae</i>	15	40.6	6	9
9	<i>Alternaria alternata</i>	-	-	7	10
10	<i>Alternaria solani</i>	-	-	1	1
11	<i>Fusarium</i>	2	5.4	2	3
12	<i>Penicillium</i>	4	10.8	-	-
	<b>Summation</b>	<b>37</b>	<b>100</b>	<b>66</b>	<b>100</b>

Fungi that appeared with low frequency, including *Alternaria*, *Penicillium* and *Fusarium*. these results are consistent with Al-shebel and Scieuces (2012), who confirmed that the fungi belonging to these genera had been isolated in a small proportion. Our results were inconsistent with Broggie *et al* (2007) which had the highest incidence of *Alternaria* and *Fusarium*. This may be due to the fact that the fungi were carried on the exterior of the seeds and were not associated with the inside of the seeds. These findings are also consistent with Choudhwy and Sinha, 1993, who indicate that *Alternaria*, *Penicillium* and *Fusarium* are the fungi accompanying the stored seeds.

Obsertions of isolated and contaminated fungi of stored seeds indicate that high levels of contamination with fungi, in particular *Aspergillus* spp., would be a public health threat because of the ability of the fungi to produce Aflatoxins, which is harmful to humans and which at a high dose is fatal and low dose is carcinogenic and mutagenic Bennett and Christenseu (1983).

### **Inhibitory Activity of Silver Nanoparticles against Fungs under Study by Wells diffusion method**

The results of the current study demonstrated the inhibitory effectiveness of silver nanoparticles (Table 2). by Bohr diffusion, where the effectiveness of silver nanoparticles against tested fungi was determined by measuring the diameters of inhibition determined around the perforation areas on the PDA substrate showing that the diameter of inhibition at a concentration of 15% in (11.33) mm was the strongest inhibitory concentration. On the other hand, the concentration (5%) was the least inhibitory concentration since the mean diameter for *Aspergillus niger* was (11.17) mm using silver nanoparticles. In



the case of *Aspergillus terreus*, the largest inhibition diameter was (12.83) mm to reinforcement (15%) and minimal butt to reinforcement (5%) and diameter (12.33) mm. The inhibitory effect of silver nanoparticles could be due to their large surface area in relation to their size (Kanhed et al., 2014).

This resulted in silver nanoparticles being unable to interact with the cell wall of the microorganism, resulting in an imbalance in cell permeability, unable to control the transport of phosphate through the plasma membrane, causing destruction of the cell membrane and especially with amino acids reacted SH and inhibits the activity of many enzymes by binding to the enzyme active site, disrupting the flow of energy, impairing the movement of electrons in the respiratory chain, inhibiting cytochromes and reactive oxygen production. Their interaction with DNA and RNA leads to reduced DNA replication and thus inhibits the growth of microorganisms (Kedzlora and Sobik, 2013). The results of the statistical analysis showed no significant differences between the different concentrations of silver nanoparticles tested, while the pesticide coefficient (positive control) showed a significant advantage in the effect on the tested fungi with the width of the inhibition zones in relation to the different concentrations of silver nanoparticles and the negative control coefficients showing no response to the fungi tested (Table 2)

This is consistent with the finding (Xu *et al.* 2013) that silver nanoparticles show a twofold inhibitory effect against *Aspergillus* spp. and twice that of other inhibitors. Silver nanoparticles acted as effective antifungal agents against the two yeasts and through (scanning) electron microscopy they observed changes in the membrane structure of both yeasts when interacts with silver nanoparticles, it is observed that holes in the membrane surface led to cell death as a result of these holes. (Lee *et al.* 2005). In other study on some plant pathogenic fungi such as *Monilinia* sp. and *Pyricularia*, silver nanoparticles inhibited the growth of these fungi and showed significant inhibitory activity. (Mahizadehe *et al.* 2015) reported that silver nanoparticles inhibited many plants pathogenic fungi. In their study, they were found to penetrate the surface of the mycelium, leading to exocytosis and contraction of the mycelium. Upon microscopic examination, silver nanoparticles were found to inhibit the formation of spores such as *Aspergillus fumigatus*.

**Table (2) Effect of nanoparticles on isolated fungi via wells diffusion method on SDA medium**

Plant  Treatments	Germination Percentage	
	<i>Aspergillus niger</i> Mean±SE	<i>Aspergillus terreus</i> Mean±SE
	Inhibition zone diameters (mm)	
Control	0 C	0 C
Difenostar	22.67±0.88 A	26.33±1.67 A
% 5	11.17±0.60 B	12.33±1.45 B
% 10	12.67±0.33 B	12.33±0.44 B
% 15	11.33±0.73 B	12.83±0.44 B
LSD	2.55	1.47

\*Similar letters indicate that there are no significant differences, while different letters indicate that there are significant differences at the probability level ( $P \leq 0.05$ ).

Concentrations greater than 1.5 mmol of silver nanoparticles in the isolated fungi inhibitor have a diameter of 0.68 mm. Fungi can be inactivated by silver nanoparticles because the silver nanoparticles are also capable of penetrating the fungal cell wall (Al-Wakeel 2013) because the silver nanoparticles are able to bind to the fungal cell wall and penetrate the fungal cell wall, cell wall disruption and absorption by interacts with the biological and metabolic processes inside the fungus affecting the regulation of fungal-specific proteins and enzymes, then inhibiting the fungus

### **The effect of silver nanoparticle in the laboratory on the vegetative and root system of the seeds under study**

#### **Germination percentage**

The results shown in Table (3) indicate that there are no differences in the percentage of germination between wheat seeds using different concentrations of nanoparticles under study (silver oxide). Transactions in the percentage of seed germination except for the pesticide treatment (positive control), where the germination rate was 96.6%, and this is consistent with (Yin *et al.* 2012) and Ba (Masoud and Bahwerth, 2017), where the high percentage of germination in seeds treated with nanoparticles may be attributed to the ability of seeds to absorb these particles, which in turn affects the percentage of germination (Kumari *et al.*, 2011).

**Table (3) Effect of nanoparticles on the germination rate of wheat grains**

Plant  Treatment	Germination Percentage
	SNPs
	Wheat
Control	100
Difenostar	96.6
%5	100
%10	100
%15	100
Average	99.32

These results indicate that the percentage of germination was not affected by the presence of the different concentrations of nanoparticles of silver oxide, and this was stated by the study of (Yin et al.,2012) and (Hassan and Hassan ,2020). Despite the widespread use of Difenconozol to prevent diseases caused by some fungi, it caused a decrease in the germination rate to 96.6% in the wheat seeds under study. These results were consistent with (Liu *et al.*, 2021) who indicated that plant exposure to the pesticide Difenconozol may cause oxidative stress, reduce biosynthesis, and then prevent the growth and development of wheat plant.

### **The length of the stem and root**

Table (4) show the blade lengths of wheat seeds that were treated with nanoparticles of silver. The obtained results show that the blade length in both types of seeds was not affected by the levels of concentrations of silver nanoparticles. The highest feather length was at a concentration of 15% for wheat seeds and using nano silver, which reached 1.04 and 1.71 cm, respectively for wheat seeds.

As we can see from Table (4) the superiority of the control treatment here, and this may be due to the sensitivity of wheat to nano-silver oxide and the absence of a hard cover for the seed, which allowed a large amount of nanoparticles to enter the blade, which caused toxicity to its cells and thus reduced its division leading to a short length of the blade The results of this

study were in agreement with the results of other researchers (Abou- Zeid and Moustafa, 2014) and (Salama *et al.*, 2012).

While our results were inconsistent with Bamasoud and Bahwerith (2017) in increasing the feather length of wheat seeds treated with silver nanoparticles compared to the control treatment. While (Salama *et al.*, 2012) indicated that nanoparticles can modify the physico-chemical properties of materials, which may affect the vitality of living cells. The results of the statistical analysis showed the treatment of silver nanoparticles for a concentration of 10% showed a significant superiority in its effect on the feather length of the seeds under study compared (Table 4).

As for the root length (Table 4), it is clear that the root lengths are superior in wheat seeds when treated with silver nanoparticles at a concentration of 15%, as the highest root length reached 5.03 cm. The results of the statistical analysis showed a significant superiority of all nanoparticles treatments under study in their effect on the root lengths of the seeds under study (Table 4). The increase in root lengths compared to the pesticide treatment and the control may be attributed to the increase in the rate of cell division of the apical roots of seedlings (Farooq *et al.*, 2005), through the role of Ag NPs in interfering with multiple pathways in plant cells to activate plant growth, as indicated by (Arase *et al.*, 2012) for the biosynthesis of the growth hormone indole acetic acid (IAA) stimulating root elongation, and as mentioned by (Singh *et al.*, 2015) in their study that silver is a great stimulator of plant growth. Silver nanoparticles have the ability to increase the root system as well as the ability to increase the root length and its branches and promote root growth (Mazumdar., 2014). Also, the nanoparticles can bind directly to the fungal cell membrane and penetrate it to kill spores and thus inhibit or kill the fungus (Hwang *et al.*, 2008). The treatment consisting of silver nanoparticles showed the highest dry weight value of 0.69 mg compared to the control treatment, which amounted to 0.65 mg. With no significant differences.

**Table (4) Effect of nano silver on stem and root length of germinated seeds of wheat**

Plant  Treatments	Wheat Mean $\pm$ SE	
	SNPs	
	Stem length	Root length
<b>Control</b>	1.20 $\pm$ 0.17 <b>A</b>	4.82 $\pm$ 0.22 <b>A</b>
<b>Difenostar</b>	0.38 $\pm$ 0.23 <b>B</b>	2.18 $\pm$ 1.91 <b>B</b>

<b>% 5</b>	0.41±0.12 <b>B</b>	3.35±0.68 <b>A</b>
<b>% 10</b>	0.69±0.13 <b>AB</b>	4.52±0.51 <b>A</b>
<b>% 15</b>	1.04±0.32 <b>AB</b>	5.03±0.86 <b>A</b>
<b>LSD</b>	0.516	2.52

**\*Similar letters indicate that there are no significant differences, while different ones indicate that there are significant differences at the probability level ( $P \leq 0.05$ ).**

### **Fresh and dry weight of feather**

Table (5) shows the superiority of the fresh feather weight of wheat grains treated with silver nanoparticles at a concentration of 10%, as the fresh feather weight reached 0.0119 mg. It was clear that we found that the silver nano concentration did not affect the fresh weight of the beak, as it was the highest weight. At a concentration of 10% (0.0119) and the lowest weight at a concentration of 5% was (0.0084) mg. It is clear from Table (5) that the dry weight of the feathers is higher at a concentration of 10% (0.0063) mg for wheat grains treated with nano silver, while hair dryness is lower by 5% (0.001) mg for wheat seeds treated with nano silver.

For a concentration of 10% less water loss is possible because the size of small nanoparticles changes the physical and chemical properties of the material, and thus affects the survival of living cells, causing damage to cell walls (Mazumdar, 2014). It plays an important role in increasing enzyme activity and thus increasing the rate of important secondary plant processes and reactions, which in turn leads to an increase in DNA, RND and peroxidase enzymes, and silver nanoparticles also have key roles in water capture by regulating growth, redox state and plant quality processes (Ghorban and Hatami, 2013)

Our results agree with (Hassan and Hassan, 2020), as the treatment including silver nanoparticles supports the highest dry weight value, up to (0.96) mg, compared with the method. control treatment, up to 0.65g.

The results of the statistical analysis showed that there was no significant difference between different concentrations the silver nanoparticle treatment was being investigated for their effect on fresh and dry weight of feather at a probability of 0.05

**Table (5) The effect of nanoparticles of silver on the wet and dry weight of stem germinated seeds of wheat (mg)**

Plant  Treatments	SNPs	
	stem wet weight	stem dry weight
	Wheat Mean±SE	Wheat Mean±SE
<b>Control</b>	0.0069±0.0018 <b>AB</b>	0.004±0.00007 <b>B</b>
<b>Difenostar</b>	0.0038±0.0038 <b>B</b>	0.0013±0.00033 <b>C</b>
<b>% 5</b>	0.0024±0.0007 <b>B</b>	0.001±0.00003 <b>BD</b>
<b>% 10</b>	0.0086±0.0017 <b>A</b>	0.0037±0.00006 <b>A</b>
<b>% 15</b>	0.0091±0.0008 <b>A</b>	0.0036±0.00012 <b>B</b>
<b>LSD</b>	0.0051	0.0004

Similar letters indicate that there are no significant differences, while different letters indicate that there are significant differences at the probability level ( $P \leq 0.05$ ).

### Fresh and dry weight of the root

Table No. (6) shows the superiority of the wet weight of the root of wheat seeds treated with nano-silver at a concentration of 5%, as it gave the highest fresh weight of 0.0255 mg, while the concentration of 15% of nano-silver for the same type of seeds gave the lowest fresh weight of the root of 0.0058 mg. Fresh and dry weight of rootstock in treated seeds. And when comparing the results of nanoparticle concentrations with the control and treatment with pesticides regarding the dry and fresh weight of the root, where the treatments consisting of silver nanoparticles showed the highest value for the dry weight in Table (20). As these particles can increase the length of the root and its branches and enhance its growth (Agustin ana Yuri., 2017).

In a study conducted by (Yahya and Muhammad, 2019) it showed the role of silver nanoparticles in the result of germination that reached 75% at a concentration of 2 mg / L, in addition to the ability of these nanoparticles at a concentration of 2 mg / L to reduce the inhibitory effect of polyethylene glycol (PEG) on the seeds Chickpeas *Cicer arietinum*. The results of the statistical analysis showed that there were no statistically significant differences between the different concentrations of nanoparticles under study in their effect on the wet and dry weight of carrots for the seeds under study, compared to the negative control treatment, which outperformed all treatments under study. Study at the probability level 0.05.

**Table (6) The effect of nanoparticle of silver on the wet and dry weight of root germinated seeds of wheat (mg)**

Plant  Treatments	SNPs	
	root wet weight	root dry weight
	Wheat Mean±SE	Wheat Mean±SE
<b>Control</b>	0.0157±0.0006 A	0.004±0.00007 A
<b>Difenostar</b>	0.0093±0.0080 A	0.0013±0.00033 C
<b>% 5</b>	0.0138±0.0017 A	0.0036±0.00017 A
<b>% 10</b>	0.0177±0.0023 A	0.0029±0.00029 B
<b>% 15</b>	0.0241±0.0053 A	0 D
<b>LSD</b>	0.0011	0.00053

Similar letters indicate that there are no significant differences, while different letters indicate that there are significant differences at the probability level ( $P \leq 0.05$ .)

The concentrations of nanoparticles used gave an encouraging role, as studies indicate that some minerals, including (Zn, Mg, Ni, Cu) represent nanonutrients that have an important role in plant growth, including many cellular functions, including protein production, photosynthesis and indole acetic acid metabolism. (Sinha, 2007) and (Sharma *et al.* 2021) many studies have indicated the use of silver nanoparticles in promoting seed germination and plant growth in wheat crops, but when applied at precisely defined concentrations (Jasrotia, 2018) (Kashyap and Matras, 2022), and thus can reduce crop stress conditions such as drought, salinity, and heat (Jiang, M., 2021) and pathogens can be controlled wheat due to its antifungal, antibacterial and antiviral properties (Gibała, 2021) and (Żeliszewska, and Malandrakis, 2019) as these studies found that nanosilver applied at an appropriate concentration could be equal or more effective compared to synthetic fungicides. (Aziz, 2016)

However, high doses of nano-silver can cause toxicity to plants, as nano-silver can show multiple patterns of inhibitory action against microorganisms (McShan, 2014) In addition, it can damage the structures of molecules inside cells (proteins, lipids and amino acids), which causes oxidative stress through the synthesis of reactive oxygen species (ROS) and the accumulation of free radicals (Dimkpa, 2013) and (Gibała, 2021) and (Malandrakis, 2019)

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