



Using nanoparticles to preserve the safety and health of seeds from black point disease in warehouses for six varieties of Iraqi wheat (*Triticum aestivum*)

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Received: 28 December 2025 / Accepted: 26 April 2026
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Abstract

A laboratory and warehouse study was conducted during the 2024/2025 season in the laboratories and warehouses of the Plant Protection Department of the College of Agriculture, Tikrit University/Iraq. The study included the use of silver nanoparticles and biosynthetic zinc nanoparticles from the food mushroom *Pleurotus ostreatus* A2019 for four parts: (mushroom filtrate, biomass, cold extract and Hot extract) to preserve the safety and health of seeds from black point disease for six varieties of Iraqi wheat. The results of the study showed that the two fungi, *Alternaria spp* and *Fusarium spp*, had the highest appearance rate among the isolated and diagnosed fungi, as the percentage of the two fungi reached 17.50% and 19.25%, respectively. The experimental treatment was reduced to study the effect Concentrations of silver nanoparticles and zinc nanoparticles on the fungi *Alternaria spp* and *Fusarium spp*, which cause black spot disease (cm). Treating the fungus filtrate with silver nanoparticles (AgNPs) at a concentration of 2mm gave the highest rate of inhibition, reaching 0.640cm for the fungus *Alternaria spp* and 0.520cm for the fungus *Fusarium spp* at the same concentration and the same treatment, while the highest percentage was Germination before storage for the Iraq 2 variety reached 92.25%, while the highest germination rate after storage was for the Aba variety, which reached 97.75%. As for estimating the storage infection rate based on the pathogenic fungus *Fusarium spp*, the lowest infection rate was in the treatment consisting of (AgNPs)+pesticide+fungus, as it reached 0.79% and 0.75% for the fungus *Alternaria spp* for the same treatment and the same concentration.

Keywords Triticum · Silver nanoparticles · Nanozinc · *Pleurotus ostreatus* A2019 · Black point disease

1 Introduction

The wheat crop belongs to the Poaceae (Gramineae) family, the *Triticum* genus, which is considered one of the strategic crops in particular that is grown on a wide range of agricultural lands in the world. Therefore, it is considered one of the most important grain crops in terms of production and cultivated area Ban and Zainab (2016). Its importance has increased further due to frequent food shortages, as it is considered The main source of food and its main role in global trade (Al-Janabi et al. 2025). The increase in wheat

production is a challenge to the world to meet the nutritional needs of the population because it provides approximately 20% of food calories in general or indirectly to the human body and represents about 30% of total grain production (Mohsen et al. 2018). Wheat grains are exposed to infection with many pathogens, whether in the field or during Storage process: Fungi transmitted by wheat grains are among the most important pathogens because they lead to reduced germination rates and poor quality. In addition, some fungi produce mycotoxins that affect human safety and health. The most important fungal genera that produce toxins are *Aspergillus*, *Fusarium*, and *Penicillium* (Muhammad et al. 2018). Wheat seeds transmit many fungi that cause several diseases during the storage process, including black point disease (Black points). This disease causes the affected seeds to change color and appear rotten and have black dots or sooty mold. Infection of the fetus leads to a reduction in

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the production and marketing capacity of the grain. The disease causes losses of up to 50% of stored wheat. The most common species causing this disease are those belonging to the genera *Stemphyllium*, *Alternaria*, *Helminthosporium*, *Fusarium*, and *Cladosporium* (Alloosh 2020). There is a need for non-toxic and environmentally friendly methods to combat plant diseases that affect crops as alternative materials to pesticides Abdullah and Hassan (2024). Nanotechnology has entered the field of technology, which is developing interest in biological approaches that are free from the use of toxic chemicals as by-products. Therefore, nanoparticle biosynthesis is preferred over physical and chemical means due to rapid synthesis, better control of size and shape properties, less toxicity, cost-effectiveness, and an environmentally friendly approach. There are a variety of natural sources of nano-mineral synthesis include plants, fungi, yeasts, filamentous bacteria, actinomycetes and bacteria Mariana and Renata (2019). 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. There are many reasons why fungi can be chosen as the best nanofactories compared to bacteria and plants (Mina et al. 2024). The mechanisms that lead to the production of nanoparticles by fungi are limited to using mushroom extract, which leads to the reduction of metals into metal particles, thus creating nanoparticles that have a major role in treating many plant diseases (Ballotin et al. 2016). Therefore, the study aimed to:

1. Isolation and diagnosis of fungi that infect wheat seeds in the warehouse, causing black spot disease in the warehouse.
2. Purifying the bush associated with wheat seeds for six varieties of Iraqi wheat.
3. Study the effect of concentrations of silver nanoparticles and nano-zinc oxide on the germination rate of wheat seeds for six Iraqi wheat varieties.
4. Evaluating the effectiveness of silver nanoparticles and zinc oxide nanoparticles on the two pathogenic fungi *Alternaria spp* and *Fusarium spp* that cause black spot disease in storage.

2 Materials and methods

Six varieties of Iraqi wheat (Abaa 99, Bohouth 22, Iraq 2, Al-Rasheed, Abu Ghraib and Jihan) were obtained from the Seed Technology Center of the Ministry of Higher Education in Salah al-Din, Iraq.

2.1 Laboratory experiments

2.1.1 Biosynthesis of nanoparticles

The biosynthesis process of silver nanoparticles and zinc oxide nanoparticles was carried out according to the method used by Hassan and Hassan (2020). Using the food mushroom *P. ostreatus A2019* obtained from the laboratories of the Plant Protection Department - College of Agriculture, Tikrit University, silver nanoparticles and zinc oxide nanoparticles were prepared from four parts, including mushroom filtrate, biomass, hot extract, and cold extract, with five concentrations for each part (0, 0.5, 1, 1.5, 2 mM). The food fungus *P. ostreatus A2019* inoculated into the liquid medium of potato sucrose broth (PSB) was filtered using filter paper. The biomass was separated from the filtrate and 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 separating the sediment from the eluent. The eluent was collected at a rate of 5 ml and placed in 10 ml tubes. Different concentrations of previously prepared silver nitrate and Zinc acetate solution (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 that did not contain any of the concentrations mentioned, only the fungal filtrate clearing. The tubes were incubated according to the fungal temperature at 25 °C for 72 hours in the dark.

2.1.2 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 instructions of the prepared company. Then sterilize the medium in an autoclave at a temperature of 121 °C and a pressure of 1.5 bar for 20 minutes, after which the medium was cooled to a temperature of 40 °C, then the antibiotic Ampiclox was added at a rate of 100 mg/liter of medium, and finally the medium was poured into Petri dishes and left to solidify at room temperature. After solidification, it was placed in the laboratory refrigerator.

2.1.3 Isolation and identification of fungi for six Iraqi wheat varieties%

Samples of wheat seeds stored for a period of (one year) were taken from warehouses for storing wheat seeds, at a rate of 1 kg for each variety. Wheat seeds for the six varieties were grown by placing 25 seeds in Petri dishes containing Potato Dextrose Agar (PDA) fungal growth medium, in

four replicates for each variety, Then they were placed in the incubator for 7 days at a temperature of 22 °C. Growth was monitored daily, and after the fungi grew and appeared, the fungal isolates were purified by transferring part of the growing colony to the culture medium (PDA). The purified fungi were then identified based on the fungal hyphae, colors and shapes of the conidia and their pods, according to the available taxonomic keys, and the infection rate was calculated according to the following equation:

$$\text{Incidence rate} = \frac{\text{Number of infected seeds}}{\text{Grand total}} \times 100$$

2.1.4 Isolating and purifying the bush associated with wheat seeds in storage%

The bush was isolated and purified from the wheat seeds of the six varieties, at a rate of four replicates for each replicate of 1 kg, in the purification laboratories of the Ministry of Agriculture, Seed Inspection and Certification Department (Iraq). The percentage of the bush was calculated according to the following equation:

$$\text{Percentage of bush} = \frac{\text{The weight of the bush}}{\text{Seeds 1 kg}} \times 100$$

2.1.5 Study of the effect of concentrations of silver nanoparticles and nano-zinc on the fungi *Alternaria spp* and *Fusarium spp*, which cause black spot disease (Cm)

The effect of concentrations of silver nanoparticles and zinc nanoparticles prepared for four fractions (mushroom filtrate, biomass, cold mass and hot mass) was studied on the growth of the two pathogenic fungi *Alternaria spp* and *Fusarium spp*, as they appear to have the highest infection rate in wheat seeds and are one of the most important pathogens of black spot disease in wheat. This is done by placing a piece of pathogenic fungus with a diameter of (1 cm) in the middle of dishes containing solid nutrient medium (PDA) using a cork culturer. When the fungal colony arrived at a distance of (1 cm) from the middle of the dish, the dishes were treated with different concentrations of silver nanoparticles and nano zinc (0.5, 1, 1.5, 2 mM). By making four holes at a distance of 2 cm from the fungal colony, the concentrations were placed in the holes in amounts of 0.1 ml and incubated at a temperature of 25 °C. When the mushroom growth in the control treatment (distilled water instead of silver nanoparticles and nano-zinc nanoparticles) had completed growth to the end of the dish, the distance from the end of the edge of the colony to the holes containing the silver nanoparticles and nano-zinc nanoparticles was measured in (cm).

2.1.6 The effect of silver nanoparticles and zinc nanoparticles prepared from the edible mushroom *P. ostreatus A2019* in four parts at a concentration of 2 mM on the germination process of wheat seeds before storage %

Wheat seeds of the six varieties were treated with a concentration of 2 mM of silver nanoparticles and nano-zinc particles, as it showed the highest rate of inhibition of the fungi *Alternaria spp* and *Fusarium spp* in four parts that included (mushroom filtrate and hot extracts and cold extracts and biomass) prepared from the food mushroom *P. ostreatus A2019*. The wheat seeds were soaked in the above-mentioned treatments. The tubes were left for 2 hours, after which the seeds were placed on blotting paper moistened with water (folding method), then placed in bags, and incubated at a temperature of 25 °C in the germinator. They were monitored regularly and the percentage of germination was recorded after ten days according to the following equation:

$$\text{Germination rate \%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

2.2 Inventory experiments

The storage infestation rate, wet weight, and germination rate of wheat seeds were estimated by taking pure samples (seeds) of 1 kg for each treatment and variety, and with four replicates. The wheat seeds were fumigated for 48 hours until the state of absorption was reached, taking into account the seeds were fumigated with nanoparticles of silver and nanozinc at a concentration of 2 mM, and the pesticide Krizer at the recommended concentration of The pesticide production company stored it for three months according to the following transactions:

1. ZnONPs
2. AgNPs
3. ZnONPs+Pathogenic fungus
4. AgNPs+Pathogenic fungus
5. Pesticide+Pathogenic fungus
6. ZnONPs+pesticide+Pathogenic fungus
7. AgNPs+pesticide+Pathogenic fungus
8. Pathogenic fungus
9. Control

2.2.1 Estimating the infection rate of wheat seeds in storage based on the two pathogenic fungi *Alternaria spp* and *Fusarium spp* that cause black spot disease %

After three months had passed since the samples were stored in the warehouse, while providing appropriate storage conditions, the samples were examined by planting wheat seeds for the six varieties, placing 25 seeds in four replicates in Petri dishes containing Potato Dextrose Agar (PDA) fungal growth medium, four replicates for each variety, then placed in the incubator for 7 days at a temperature of 22 °C. The seeds were monitored daily, and after the fungi grew and appeared, the fungal isolates were purified by transferring a portion of the growing colony to the culture medium (PDA), and the infection rate was calculated according to the following equation:

$$\text{Incidence rate} = \frac{\text{Number of infected seeds}}{\text{Grand total}} \times 100$$

2.2.2 Calculate dry weight (g)

The dry weight of the plant was calculated after drying the wheat seeds for three days by exposing them to sunlight in three repetitions. The dry weight of the plant roots was calculated after the weight was stabilized using a sensitive balance for each of the experimental treatments separately.

2.2.3 Effect of silver nanoparticles and zinc nanoparticles prepared from the edible mushroom *P. ostreatus A2019* in four parts at a concentration of 2 mM on the germination process of wheat seeds after storage %

The germination percentage after storage was calculated according to the above-mentioned treatments, by placing the seeds on blotting paper moistened with water (folding method) in the amount of 100 seeds for four replicates, then they were placed in bags, and incubated at a temperature of 25 °C in the germination facility. They were monitored

regularly, and the germination percentage was recorded after ten days according to the following equation:

$$\text{Germination rate \%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

2.3 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 level of 0.05 Al-Rawi and Abdul Aziz (2000).

3 Results

3.1 Isolation and diagnosis of pathogenic fungi causing black point disease for six Iraqi wheat varieties%

The results are shown in Table 1 for the isolation and diagnosis of the pathogenic fungi that cause black point disease ((Black points) for six varieties of Iraqi wheat. The percentage of infection with the fungus *Fusarium spp* reached 13.66%, while the percentage of infection with the fungus reached 14.91%, with significant differences with the next fungus, *Altrnaria spp*. The infection rate reached 13.66%, while the lowest infection rate was In the fungi isolated in the fungus *Aspergillus spp*, it reached 5.25%, with significant differences with the fungi *Fusarium spp* and *Altrnaria spp* (see Fig. 1).

Table 1 Isolation and diagnosis of pathogenic fungi causing black point disease (Black points %)

Isolated fungi	Items						Rate
	Abaa 99	Bohouth 22	Iraq 2	Al-Rasheed	Abu Ghraib	Jihan	
<i>Helminthosporium spp</i>	H-L8.00	H-M7.50	I-O7.00	GHI 9.00	I-P6.50	I-P6.75	7.45
<i>Stemphyllium spp</i>	J-Q6.00	I-P6.50	PQ4.25	N-Q4.75	O-Q4.50	M-Q5.25	D5.20
<i>Cladosporium spp</i>	I-N7.25	I-O7.00	H-L8.50	I-P6.50	H-L8.00	L-Q5.75	7.16
<i>Altrnaria spp</i>	AB17.50	AB17.25	EFG11.00	DEF12.25	FGH10.00	CD14.00	B13.66
<i>Fusarium spp</i>	A19.25	DEF12.25	CDE13.50	DEF11.75	AB17.00	BC15.75	A14.91
<i>Rhizoctania spp</i>	GHI9.00	H-L8.00	G-J8.50	GHI 9.00	K-Q5.75	GHIJ8.25	C8.08
<i>Aspergillus spp</i>	K-Q5.75	O-Q4.50	H-M7.75	Q3.50	O-Q4.50	L-Q5.50	5.25
<i>Penicilium SPP</i>	M-Q5.25	N-Q4.75	H-L8.00	PQ4.25	I-P6.50	N-Q4.75	5.58
Rate	A9.75	8.46	B8.56	D7.62	7.84	8.15	8.4

Least significant difference L.S.D._{0.05} for fungi: 1.09 for taxa: 0.947 for overlap: 2.037

Fig. 1 *Fusarium* spp and *Alternaria* spp fungi after the isolation process (Paragraph 3.1 follows)



Table 2 Isolation of bushes associated with wheat seeds in storage %

Items							
Type of bush	Abaa 99	Bohouth 22	Iraq 2	Al-Rasheed	Abu Ghraib	Jihan	Rate
barley	^A 2.82	^{AB} 2.72	^{ABC} 2.70	^{ABCD} 2.65	^{ABCD} 2.64	^{ABCD} 2.62	^A 2.69
Wild barley	2.17	2.15	1.87	1.52	1.82	1.6	1.85
baker	2.12	1.3	2.13	1.65	1.4	2.26	1.81
Handaquq	1.9	2.07	1.45	2.16	1.35	2.02	1.82
Wild oats	2.2	1.9	^{A-F} 2.42	2.14	2.04	1.5	^B 2.03
Fajila	1.95	1.47	1.85	1.65	2.4	1.27	1.76
Kalgan	2.16	^{A-E} 2.55	1.75	1.56	1.52	1.82	1.89
Other	1.87	1.5	2.12	2.27	1.65	^M 1.25	1.77
Rate	^A 2.14	1.95	^B 2.03	1.95	1.85	1.79	1.95

Least significant difference L.S.D._{0.05} for bush: 0.290 for varieties: 0.251 for overlap: 0.541

3.2 Isolating and purifying the bush associated with wheat seeds in storage%

Table 2 shows the isolation and purification of the bushes associated with wheat seeds in the warehouse. The percentage of bush isolation (barley) was superior to the rest of the treatments, as the highest percentage reached 2.69%, followed by the bush treatment (Handaquq), which reached 2.03%, with significant differences between the two treatments, while the lowest percentage of bush isolation was in the bush treatment (Fajila), which amounted to 1.76%, with significant differences with the treatment. The bush (barley) and (handaquq),

3.3 Study of the effect of concentrations of silver nanoparticles and nano-zinc on the fungi *Alternaria* spp and *Fusarium* spp, which cause black spot disease (Cm)

The results of the study of the effect of concentrations of silver nanoparticles and nano-zinc on the fungi *Alternaria* spp and *Fusarium* spp, which cause black point disease (cm), in Tables 3 and 4 showed the superiority of the treatment of mushroom filtrate prepared from silver nanoparticles

(AgNPs) when treating the fungus *Alternaria* spp, as the highest rate of inhibition reached 0.379 cm compared with the treatment followed by it. The mushroom filtrate prepared from zinc nanoparticles (ZnNPs) reached 0.365 cm, with significant differences compared to the biomass zinc nanoparticles (ZnNPs) treatment, which showed the lowest rate of inhibition, reaching 0.305 cm, with significant differences with the two treatments of mushroom filtrate from silver nanoparticles and the mushroom filtrate from the zinc nanoparticles treatment.

Table 4, the nano-treatment prepared from mushroom filtrate of zinc nanoparticles (ZnNPs), reaching 0.285 cm, excelled over the rest of the treatments, followed by the nano-treatment prepared from silver nanoparticles of mushroom filtrate, reaching 0.283 cm. There were no significant differences, while the nano-treatment prepared from silver nanoparticles of mushroom biomass had the lowest rate of inhibition, reaching 0.215 cm, with significant differences with the two treatments of mushroom filtrate for silver nanoparticles and mushroom filtrate for nano-zinc. As for the interference, the treatment of mushroom filtrate made of silver nanoparticles (AgNPs) at a concentration of 2 mM was superior, reaching 0.520 cm, with significant differences with the treatment of zinc biomass nanoparticles (ZnNPs)

Table 3 Effect of silver nanoparticle and zinc nanoparticle concentrations on *Alternaria* spp (cm)

Nanoparticle Transactions	Concentrations mM					Rate
	0	0.5	1	1.5	2	
filtrate ZnONPs	0	^{MN} 0.337	^{IJ} 0.400	^{DE} 0.480	^A 0.615	^B 0.365
Hot extract ZnONPs	0	^P 0.295	^{JK} 0.377	^{FG} 0.452	^B 0.545	^D 0.333
Cold extract ZnONPs	0	^{NO} 0.322	^{JK} 0.387	^{EF} 0.470	^C 0.510	^{CD} 0.337
Biomass ZnONPs	0	^Q 0.247	^{LM} 0.350	^{GH} 0.430	^{CD} 0.500	^F 0.305
filtrate AgNPs	0	^{LMN} 0.340	^{HI} 0.417	^{CDE} 0.495	^A 0.640	^A 0.379
Hot extract AgNPs	0	^{MNO} 0.327	^{IJ} 0.392	^{EF} 0.475	^B 0.555	^C 0.349
Cold extract AgNPs	0	^{OP} 0.310	^{JK} 0.385	^{FG} 0.455	^C 0.525	^D 0.335
Biomass AgNPs	0	^P 0.295	^{KL} 0.365	^{GH} 0.435	^{CD} 0.500	^E 0.319
Rate	0	^D 0.309	^C 0.384	^B 0.461	^A 0.548	0.425

Least significant difference L.S.D._{0.05} for coefficients: 0.011 for concentrations: 0.009 for interference: 0.026

Table 4 Effect of silver nanoparticle and zinc nanoparticle concentrations on *Fusarium* spp (cm)

Nanoparticle Transactions	Concentrations mM					Rate
	0	0.5	1	1.5	2	
filtrate ZnONPs	0	^{LM} 0.212	^G 0.342	^F 0.390	^B 0.485	^A 0.285
Hot extract ZnONPs	0	^{MNO} 0.182	^{KL} 0.238	^{GH} 0.330	^{CDE} 0.427	^{CD} 0.235
Cold extract ZnONPs	0	^{NO} 0.177	^{KL} 0.237	^G 0.342	^C 0.447	^C 0.240
Biomass ZnONPs	0	^O 0.175	^{KL} 0.230	^I 0.292	^{C-F} 0.425	^D 0.224
filtrate AgNPs	0	^{LM} 0.215	^{IJ} 0.282	^{EF} 0.402	^A 0.520	^{AB} 0.283
Hot extract AgNPs	0	^{LMN} 0.210	^{JK} 0.255	^G 0.345	^B 0.482	^B 0.258
Cold extract AgNPs	0	^{MNO} 0.197	^{KL} 0.234	^{GH} 0.332	^{CD} 0.442	^C 0.241
Biomass AgNPs	0	^P 0.1300	^{KL} 0.235	^{HI} 0.300	^{DEF} 0.412	^F 0.215
Rate	0	^D 0.171	^C 0.256	^B 0.341	^A 0.455	0.244

Least significant difference L.S.D._{0.05} for coefficients: 0.014 for concentrations: 0.011 for interference: 0.032

at a concentration of 0.5 mM, which had the lowest percentage of inhibition (see Fig. 2.)

3.4 Effect of concentrations of silver nanoparticles and zinc nanoparticles prepared from the edible mushroom *P. ostreatus* A2019 in four parts at a concentration of 2 mM on the germination process of wheat seeds before storage %

Table 5: The effect of silver nanoparticles and nano-zinc prepared from the edible mushroom *P. ostreatus* A2019 for four parts at a concentration of 2 mM on the germination process of wheat seeds before storage% indicates that the silver nanoparticles (AgNPs) leachate treatment was superior to the rest of the treatments, reaching 94.87%, with significant differences with the nano-zinc biomass treatment. Which had the lowest germination rate, reaching 87.20% compared to the control treatment, which reached 92.00%. As for the varieties, the Iraq 2 variety outperformed the rest of the varieties in the wheat seed germination rate, reaching 92.25%, with no significant differences with the B99 variety, reaching 92.05%, while the Jihan variety had the lowest seed germination rate, reaching 92.05%. 90.83%, with significant differences With the varieties Iraq 2 and Ibaa 99, with regard to the interaction, the treatment of mushroom filtrate prepared from silver nanoparticles of the

variety Bohuth 22 had the highest germination rate, reaching 96.50%, with significant differences with the treatment of biomass prepared from nano-zinc of the variety Jihan, which reached 85.00% compared to the control treatment of the variety Abaa 99, which reached 93.25%.

3.5 The effect of silver nanoparticles and zinc nanoparticles prepared from the fungus *P. ostreatus* 2019A on the percentage of *A. officinalis* infection (%)

3.5.1 The effect of silver nanoparticles and zinc nanoparticles prepared from the fungus *P. ostreatus* a 2019 on the rate of storage infection of six Iraqi wheat varieties under conditions of infection with the pathogenic fungus *Alternaria* spp %

The results of Table 6, for estimating the rate of infection of the storage tank based on the pathogenic fungi *Alternaria* spp and *Fusarium* spp, which cause black point disease, show a % reduction in the infection rate to the level of 0 in the treatment of nanozinc (ZnONPs) and the treatment of nanoparticles (AgNPs), while the treatment of nanozinc with the pathogenic fungus (ZnONPs+pathogenic fungi) showed a reduction in the infection rate to the level of 5.08%. There were no significant differences with the treatment of silver

Fig. 2 Effect of silver nanoparticles and zinc nanoparticles on the growth of *Fusarium* spp and *Alternaria* spp fungi (by etching method) (Paragraph 3.3 follows)

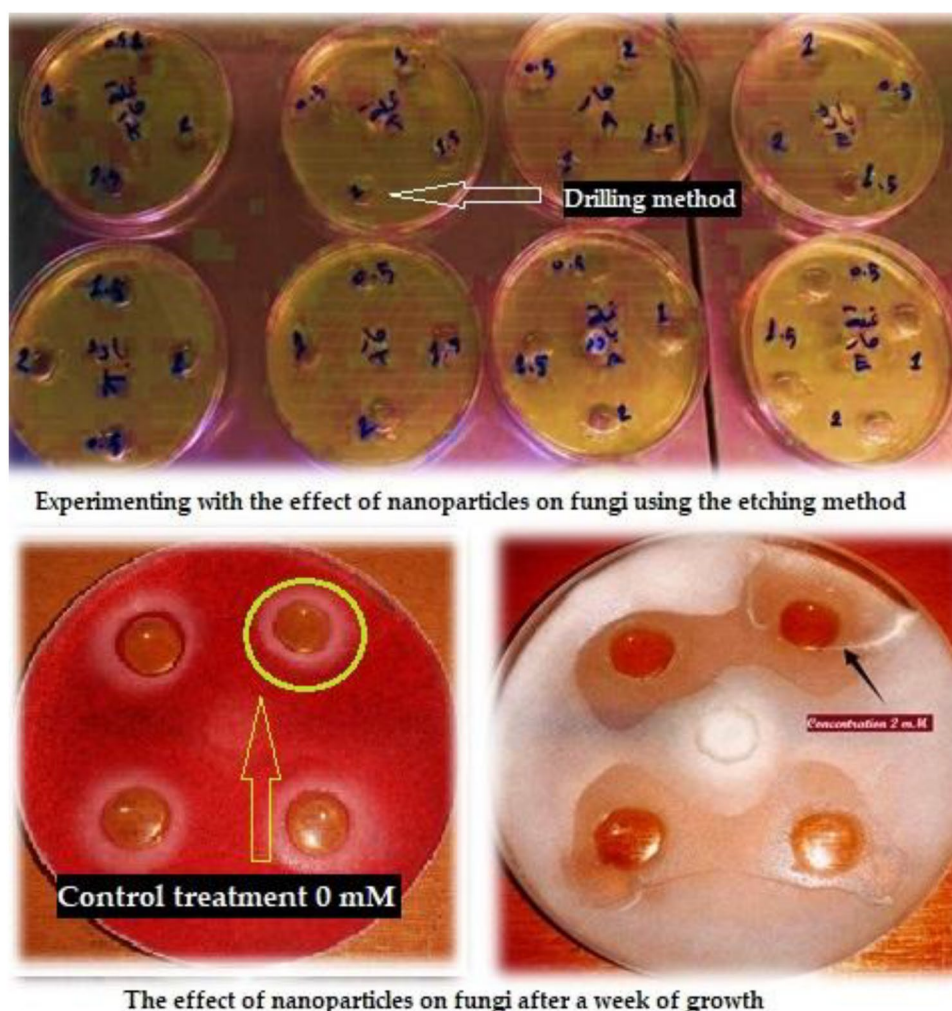


Table 5 Effect of concentrations of silver nanoparticles and zinc nanoparticles prepared from the edible mushroom *P. ostreatus A2019* in four parts at a concentration of 2 mM on the germination process of wheat seeds before storage %

	Items						
Nanoparticle Transactions	Abaa 99	Bohouth 22	Iraq 2	Al-Rasheed	Abu Ghraib	Jihan	Rate
filtrate ZnONPs	94.25	94.00	93.75	93.75	93.50	93.25	93.75
Hot extract ZnONPs	92.00	91.75	91.50	90.75	90.00	91.00	91.16
Cold extract ZnONPs	92.75	91.50	92.50	92.00	91.75	89.75	91.70
Biomass ZnONPs	89.25	85.75	88.50	86.50	88.25	^N 85.00	87.20
filtrate AgNPs	95.00	^A 96.50	94.75	94.50	94.25	94.25	94.87
Hot extract AgNPs	93.00	92.75	93.00	92.00	91.75	91.50	92.33
Cold extract AgNPs	93.25	93.00	92.75	92.75	92.25	92.50	92.75
Biomass AgNPs	85.75	89.25	90.00	89.00	89.50	86.50	88.29
control	93.25	90.25	93.50	90.75	90.50	93.75	92.00
Rate	92.05	91.63	92.25	91.33	91.30	90.83	91.56

Least significant difference L.S.D._{0.05} for transactions: 1.38 for items: 1.20 for overlap: 2.58

nanoparticles with mushrooms (AgNPs + pathogenic fungi), which recorded the lowest fungal infection rate, reaching 2.00% compared to the control treatment, which recorded 0% storage infection rate, while it was recorded on the storage treatment rate of mushroom infection, which amounted

to 66.33%. Although there were significant differences with the rest of the treatments, as for the varieties, the Abaa variety recorded the highest storage infection rate, reaching 11.30%, while the Jehan variety recorded the lowest storage infection rate, reaching 6.91%. There were significant

Table 6 The effect of silver nanoparticles and zinc nanoparticles prepared from the fungus *P.ostreatus* 2019A on the infection rate of storage under conditions of infection with the pathogenic fungus *Alternaria* spp %

Transactions	Items						Rate
	Abaa 99	Bohouth 22	Iraq 2	Al-Rasheed	Abu Ghraib	Jihan	
ZnONPs	0	0	0	0	0	0	0
(AgNP _s)	0	0	0	0	0	0	0
ZnONPs + pathogenic fungi	EFG 5.50	EFG 5.25	EFG 5.25	EFG 4.25	EFG 5.00	EFG 5.25	5.08
AgNPs + pathogenic fungi	3.75	1.50	2.00	3.50	3.50	3.75	2.00
Pesticide + pathogenic fungus	E8.25	EF7.75	EFG 7.00	EFG 6.75	EFG 7.00	EFG7.25	7.33
ZnONPs + pesticide + pathogenic fungus	1.50	0.50	0.75	0.75	0.75	1.00	0.87
AgNPs + pesticide + pathogenic fungus	G0.25	0.75	1.50	1.00	0.50	0.50	0.75
pathogenic fungus	^A 82.50	^A 79.50	^B 69.75	^C 57.75	^{BC} 64.00	^D 44.50	66.33
control	0	0	0	0	0	0	0
Rate	11.30	10.58	9.58	8.22	8.97	6.91	9.15

Least significant difference L.S.D. _{0.05} for coefficients: 3.133 for items: 2.558 for overlap: 5.691

Table 7 The effect of silver nanoparticles and zinc nanoparticles prepared from the fungus *P.ostreatus* A2019 on the infection rate of storage under conditions of infection with the pathogenic fungus *Fusarium* spp %

Transactions	Items						Rate
	Abaa 99	Bohouth 22	Iraq 2	Al-Rasheed	Abu Ghraib	Jihan	
ZnONPs	0	0	0	0	0	0	0
(AgNP _s)	0	0	0	0	0	0	0
ZnONPs + pathogenic fungi	7.75	6.75	7.75	7.50	6.50	6.75	6.08
AgNPs + pathogenic fungi	6.25	4.50	6.25	4.75	4.50	4.00	5.04
Pesticide + pathogenic fungus	^D 11.75	^{DE} 11.50	^{DEF} 10.50	^{D-G} 8.75	^{D-G} 9.00	^{D-H} 8.50	10.00
ZnONPs + pesticide + pathogenic fungus	2.75	2.25	1.25	1.75	1.50	1.25	1.79
AgNPs + pesticide + pathogenic fungus	1.00	0.75	1.25	0.75	^K 0.50	^K 0.50	0.79
pathogenic fungus	^A 78.00	^A 77.50	^B 67.00	^B 60.75	^A 74.25	^C 49.50	67.83
control	0	0	0	0	0	0	0
Rate	11.94	11.47	10.44	9.36	10.69	7.83	10.17

Least significant difference L.S.D. 0.05 for coefficients: 2.827 for items: 2.308 for overlap: 5.133

differences with the Abaa variety in terms of interaction, as the fungus treatment of the Abaa variety recorded the highest storage infection rate, reaching 82.50%, with significant differences with the rest of the treatments. While the lowest storage infection rate was recorded for the two treatments of nanozinc and silver nanoparticles for all types, as it reached 0% storage infection

3.5.2 The effect of silver nanoparticles and zinc nanoparticles prepared from the fungus *P.ostreatus* a 2019 on the rate of storage infection of six Iraqi wheat varieties under conditions of infection with the pathogenic fungus *Fusarium* spp %

As for Table 7, the mushroom treatment recorded the highest storage infection rate, reaching 67.83%, with significant differences with the rest of the treatments, while the lowest storage infection rate was in the two treatments of zinc nanoparticles (ZnONPs) and silver nanoparticles, as each of them recorded 0. % Store infection compared to the two treatments of nano zinc and pathogenic fungus (ZnONPs+pathogenic fungus) was recorded at 6.08%. As for the varieties,

the Abaa variety recorded the highest rate of storage infection, reaching 11.94%, followed by the type Bohouth 22, as it recorded 11.47%, with no significant differences compared to the variety Jihan, which recorded the lowest rate of storage infection, reaching 7.83. %, with significant differences with the rest of the varieties As for the interaction, the fungus treatment of the Abaa variety recorded the highest storage infection rate, reaching 78.00%, compared to the two treatments of nano-zinc and silver nanoparticles, each of which had a 0% storage infection rate, while the treatments of ZnONPs+pesticide+pathogenic fungus for the Jihan variety, and (AgNPs)+pesticide+pathogenic fungus for the Iraq 2 variety, reduced the lowest storage infection rate, reaching 1.25. 0.50%, respectively (see Fig. 3).

3.6 The effect of silver nanoparticles and zinc nanoparticles prepared from the fungus *P.ostreatus* 2019A on dry weight (g) under conditions of infection with the pathogenic fungus *Fusarium* spp

The results in Table 8, of the effect of silver nanoparticles and nano-zinc prepared from the fungus A2019 *P.ostreatus*

Fig. 3 Effect of silver nanoparticles and zinc nanoparticles on *Fusarium* fungi (Paragraph 3.5 follows)

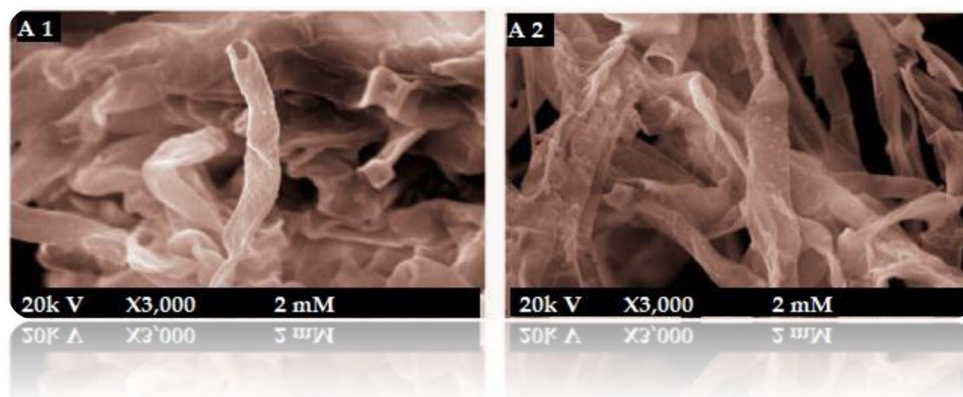


Table 8 Effect of silver nanoparticles and zinc nanoparticles prepared from the fungus *P.ostreatus* A2019 on the dry weight (g) of six Iraqi wheat varieties under conditions of infection with the pathogenic fungus *Fusarium* spp

Transactions	Items						Rate
	Abaa 99	Bohouth 22	Iraq 2	Al-Rasheed	Abu Ghraib	Jihan	
ZnONPs	^A 0.547	0.525	0.500	0.490	0.470	0.452	^A 0.497
(AgNP _s)	^A 0.540	0.522	0.492	0.485	0.457	0.454	0.491
ZnONPs+pathogenic fungi	0.407	0.387	0.380	0.375	0.371	0.372	0.382
AgNPs+pathogenic fungi	0.390	0.382	0.377	0.373	0.3750	0.370	0.377
Pesticide+pathogenic fungus	0.365	0.362	0.367	0.367	0.362	0.365	0.364
ZnONPs+pesticide+pathogenic fungus	0.452	0.442	0.437	0.425	0.422	0.417	0.432
AgNPs+pesticide+pathogenic fungus	0.447	0.440	0.435	0.425	0.420	0.412	0.429
pathogenic fungus	0.237	0.257	0.267	^U 0.232	0.260	0.245	^E 0.249
control	0.510	0.493	0.514	0.532	0.506	0.525	0.496
Rate	^A 0.432	^E 0.371	0.418	0.411	0.404	0.401	0.406

Least significant difference L.S.D. _{0.05} for coefficients: 0.016 for items: 0.013 for overlap: 0.029

on the dry weight (g) of six Iraqi wheat varieties under conditions of infection with the pathogenic fungus *Fusarium* spp indicate that the nano-zinc treatment was superior to the rest of the treatments, reaching 0.497 g compared to the silver nanoparticle treatment, which amounted to 0.491 g. There were no significant differences, while the pathogenic fungi treatment had the lowest dry weight of seeds, reaching 0.249 grams, with significant differences with the ZnONPs+pesticide+pathogenic fungus, reaching 0.432 grams. As for the varieties, the Abaa variety outperformed the rest of the varieties, reaching 0.432 grams for the dry weight of seeds, with significant differences with the rest of the varieties. While the Bohuth 22 cultivar recorded the lowest dry weight, reaching 0.371 g. As for the intervention, the nano-zinc treatment of the B-variety recorded the highest dry weight, reaching 0.547 g, with significant differences with the pathogenic fungi treatment, which recorded the lowest seed dry weight, reaching 0.232 g.

3.7 Effect of silver nanoparticles and zinc nanoparticles prepared from the fungus *P.ostreatus* A2019 on seed germination percentage (%)

The results in Table 9, show the effect of silver nanoparticles prepared from the 2019 fungus *P.ostreatus* A on the seed germination rate (%) after storage for six Iraqi wheat varieties under conditions of infection with the pathogenic fungus *Alternaria* spp. The zinc nanoparticles (ZnONPs) treatment outperformed the germination rate over the rest of the treatments, reaching 96.08%, with significant differences With the pathogenic fungus treatment, which recorded the lowest germination rate, reaching 79.41%, compared to the control treatment, which recorded a 94.50% germination rate, while the Jehan variety recorded the highest germination rate, reaching 93.18%, compared to the Abu Ghraib variety, which recorded the lowest germination rate, reaching 91.00%, with significant differences. As for the interference, the silver nanoparticles (AgNPs) treatment of the Bohuth 22 variety recorded the highest germination rate, reaching 97.75%, with significant differences with the pathogenic fungus treatment of the Iraq 2 variety, which recorded the lowest germination rate, reaching 71.50%.

Table 9 Effect of silver nanoparticles and zinc nanoparticles prepared from the fungus *P. ostreatus* a 2019 on the germination rate of seeds under conditions of infection with the fungus *Alternaria* spp

Transactions	Items						Rate
	Abaa 99	Bohouth 22	Iraq 2	Al-Rasheed	Abu Ghraib	Jihan	
ZnONPs	97.50	96.00	95.00	96.25	95.25	96.50	^A 96.08
(AgNP _s)	96.50	^A 97.75	94.50	95.75	94.50	96.00	95.83
ZnONPs + pathogenic fungi	93.50	92.75	93.75	93.25	94.00	93.00	93.41
AgNPs + pathogenic fungi	94.00	92.50	93.25	93.50	93.00	93.70	93.32
Pesticide + pathogenic fungus	92.25	90.50	93.75	92.00	92.75	92.25	92.25
ZnONPs + pesticide + pathogenic fungus	92.75	91.75	94.00	92.00	92.50	93.75	92.79
AgNPs + pesticide + pathogenic fungus	92.00	91.50	92.50	96.00	92.25	91.75	92.66
pathogenic fungus	74.00	79.75	^Q 71.50	75.25	72.00	86.00	^E 79.41
control	96.25	93.25	93.75	95.25	92.75	95.75	94.50
Rate	92.08	91.75	91.33	92.13	^D 91.00	^A 93.18	91.91

Least significant difference L.S.D. _{0.05} for coefficients: 1.374 for items: 1.122 for overlap: 2.496

Table 10 Effect of silver nanoparticles and zinc nanoparticles prepared from the fungus *P.ostreatus* A2019 on the germination rate of seeds under conditions of infection with the fungus *Fusarium* spp

Transactions	Items						Rate
	Abaa 99	Bohouth 22	Iraq 2	Al-Rasheed	Abu Ghraib	Jihan	
ZnONPs	^A 98.00	93.50	96.50	90.75	93.75	97.25	^A 94.95
(AgNP _s)	93.50	90.75	90.50	94.00	96.00	95.25	93.33
ZnONPs + pathogenic fungi	93.25	93.25	94.25	91.50	93.25	90.50	92.66
AgNPs + pathogenic fungi	95.00	90.75	96.25	97.25	91.50	93.75	94.08
Pesticide + pathogenic fungus	96.75	95.50	90.50	94.50	94.75	92.25	94.04
ZnONPs + pesticide + pathogenic fungus	93.75	94.25	92.25	90.25	91.75	92.50	92.45
AgNPs + pesticide + pathogenic fungus	94.75	90.50	96.50	97.50	92.00	95.00	94.37
pathogenic fungus	74.00	^Q 69.25	81.50	76.50	73.25	77.25	^E 75.29
control	96.25	93.25	94.25	92.75	90.75	93.25	93.41
Rate	^A 92.80	^D 90.11	92.50	91.63	^D 90.76	91.88	91.62

Least significant difference L.S.D. _{0.05} for coefficients: 1.283 for items: 1.154 for overlap: 2.437

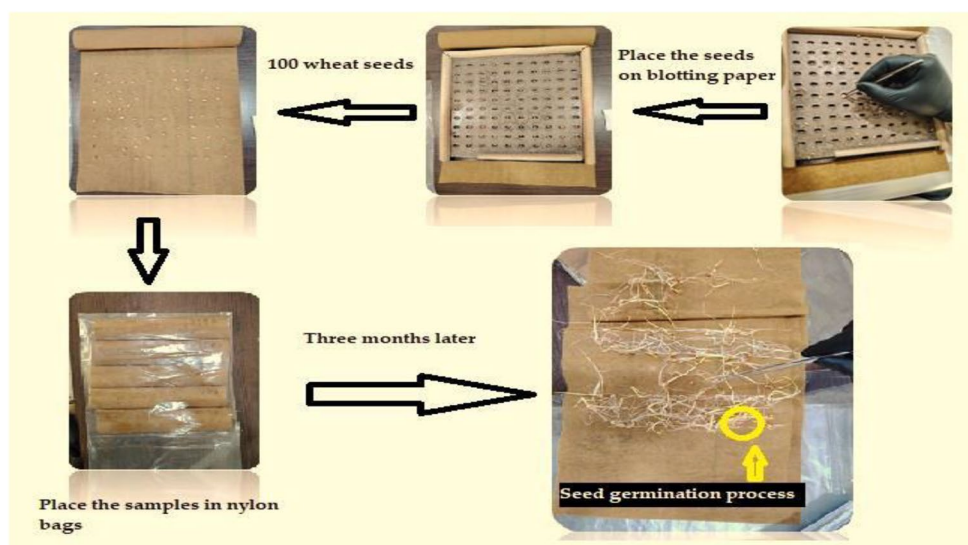
As for Table 10, the nanozinc treatment (ZnONPs) showed the highest germination rate, reaching 94.95%, with significant differences with the pathogenic fungus treatment, which recorded the lowest germination rate, reaching 75.29%. The Abaa variety recorded the highest germination rate, reaching 92.80%, with significant differences with the Abu Ghraib variety, which recorded the lowest germination rate, as reached 90.76%, As for the interaction, the zinc nanoparticles (ZnONPs) treatment of the Abaa 99 variety recorded the highest germination rate, reaching 98.00%, with significant differences with the treatment of the pathogenic fungus of the Bohuth 22 variety compared to the control treatment of the Aaba variety, which reached 96.25% (see Fig. 4).

4 Discussions

The reason for the presence of the isolated fungi in wheat seeds is that the pathogenic fungi are highly virulent in attacking their plant hosts, given the appropriate storage conditions. The fungi showed high virulence towards the

seeds through direct penetration due to mechanical pressure through the appressorium (Ahmed et al. 2022). Which in turn works to penetrate the skin and cuticle cells mechanically as a result of the pressure of the fungus on the skin It also produces cell wall-degrading enzymes and 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 Mohanad and Moafak (2022). The reason for the presence of bushes with wheat seeds in the warehouse is due to the poor purification of wheat seeds after harvest in special purification laboratories (Bilal et al. 2025). Also, the presence of bushes in the field may help them widely due to their spread with wheat seeds Especially since there are many means by which the jungle spreads, some of it is transmitted by seeds and wind, such as halva, or by water, such as sorrel, or by animals and humans, such as slime, or by agricultural machines, machinery, and seed residues after cleaning Ahmed and Hadeel (2021). Jungle plants cause serious losses in all agricultural fields and non-agricultural lands. However, due to their large presence and everywhere,

Fig. 4 Method of planting and examining seed germination after three months (Paragraph 3.7 follows)



people are accustomed to seeing them daily, and therefore they do not appreciate the greatness of the losses caused by these pests. Studies in many countries have shown that the losses caused by jungle plants may exceed the total losses caused by insects and diseases combined (Nadeem et al. 2019). The reason for the inhibition of the two fungi by concentrations of nanoparticles of silver and nanoparticles of zinc is due to their ability to affect the DNA of the pathogenic fungus by causing the DNA to lose its ability to replicate and replicate, which leads to a defect in the process of cloning the DNA strand (Sonbol et al. 2025). Silver nanoparticles and nano-zinc particles have the ability to damage the walls of the fungal hyphae, which causes dissolution of the plasma membranes of the fungi. Such damage occurs on the surface of the fungal hyphae, which leads to a defect in the plasma membranes, causing the release of internal cellular materials and thus shrinking and destroying the fungal hyphae. It also has an effective role in affecting mushroom proteins, which leads to the destruction of the protein in the fungal cell wall. They have the ability to adhere to the fungal cell walls and then penetrate, analyze and absorb the cell walls through interaction with the metabolic and biological processes inside the fungus, which leads to influencing the regulatory processes of the mushroom's proteins and enzymes and thus inhibiting the fungus Hassan and Hassan (2021). The reason for the low rate of *A. officinale* infection in the treatment consisting of the chemical pesticide Clazir, nanoparticles, and nanozinc is due to the presence of the pathogenic fungus. Nanoparticles and nano-zinc have an essential role in the process of activating the chemical pesticide Clazir and a high increase in the toxicity of the pesticide, which led to the inhibition and destruction of the fungus, in addition to the unique properties that the nanoparticles carry towards inhibiting the fungus by affecting the cell protein and DNA of the fungus

That is, the existence of a state of compatibility and synergy between the chemical pesticide and nanoparticles, which led to a reduction in the severity of root infection, with the absence of any case of chemical conflict between the chemical pesticide, nanoparticles, and nano-zinc (Sharma et al. 2022). The results of the effect of silver nanoparticles and nano-zinc on the germination rate are explained by the fact that the concentrations of silver nanoparticles and nano-zinc did not have any negative effect on the germination process of wheat seeds in all fungal treatments of silver nanoparticles and nano-zinc. Nanoparticles also have the ability to increase the water content, which in turn leads to increased moisture content. In addition, nanoparticles work to increase cell division, which leads to an increase in the germination rate in the plant. The reasons for the decrease or increase in germination may also be attributed to other compounds present within the fungal filtrate, biomass, or both hot and cold extracts, and such compounds (alkaloids, phenols, enzymes) (Malik et al. 2024). The presence of such compounds or their interaction with concentrations of nanoparticles may encourage or inhibit seed germination. 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 (Wuttipong et al. 2017). The dry weight results explain that silver nanoparticles play an important role in the process of increasing enzymatic activity and thus increasing the speed of vital processes and reactions in plants due to the large surface area they possess when the particles are at the nano level, which in turn leads to an increase in cell divisions, and biochemical reactions due to the increase in DNA, RNA, Hassan and Hassan (2026) and peroxide enzymes. Silver nanoparticles also play a major role in the water capture process by modifying the state of growth, oxidation,

and processes in plant quality. The lowest value of the dry weight in the treatment of pathogenic fungi is explained by the fact that the pathogenic fungus caused damage to the tissues of the root system, as well as directly injuring and wounding the roots. Moreover, it works to prevent the formation of root hairs, which in turn reduces the average value of the dry weight in the plant (Lauris et al. 2022).

5 Conclusions

The results of the study demonstrated the ability of fungi to biosynthesise silver nanoparticles and nano-zinc nanoparticles and the high efficiency of these materials in inhibiting plant pathogenic fungi, especially pathogenic fungi that infect wheat crops in storage at a concentration of 2 mM through their effect on the fungus protein and DNA. The study also showed that nanomaterials do not have a negative effect on the germination rate or on the dry weight of wheat seeds before and after storage. Nanoparticles, after mixing them with chemical pesticides, effectively contributed to reducing the rate of storage infection with black point disease, which affects wheat seeds after storing the seeds for three months, as they reduced the rate of infection with the *Fusarium* fungus by 0.50% and by the *Alternaria* fungus by 0.25%. The use of biosynthetic nanoparticles from fungi contributes effectively to reducing fungal infection in the warehouse and the field, as the study demonstrated the high ability of these materials to reduce the rate of fungal infection. The study also showed that there are no negative effects on human health through the use of food grains for human consumption, which prompts researchers to use them as an effective alternative to chemical pesticides.

Acknowledgements I extend my thanks and appreciation to the Seed Inspection and Certification Department of the Iraqi Ministry of Agriculture for providing seeds of all wheat varieties.

Author contributions All contributions to this scientific research are by the researcher himself.

Funding There is no funding from any institution or company, but rather self-funding by the researcher.

Data availability The datasets generated during and/or analysed during the current study are available from the author on reasonable request.

Declarations

Ethics approval Not applicable

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The author declares no competing interests.

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