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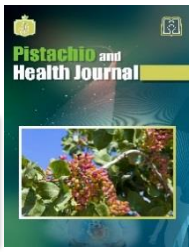

ORIGINAL ARTICLE

An evaluation of antifungal and antitoxigenicity effects of Ag/Zn and Ag nanoparticles on *Aspergillus parasiticus* growth and aflatoxin production

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Background: Aspergillus parasiticus is one of the primary sources of aflatoxin contamination in agricultural crops, especially nuts. Aflatoxin contamination is one of the most challenging problems in the production and exportation of pistachios.

Materials and Methods: In this study, the antifungal effects of the silver-zinc mixture and silver nanoparticles on the mycelia growth, spore germination and aflatoxin production of *Aspergillus parasiticus* have been evaluated.

Results: The findings of the current study showed that the concentrations of 1250 ppm of Ag/Zn and 100-200 ppm of Ag nanoparticles inhibit mycelia growth and the spore germination of *Aspergillus parasiticus* in liquid and solid media significantly, but the concentration of 25 ppm of both nanoparticles has no inhibitory effects on the *A. pararsiticus* growth parameter. The results showed that the rate of inhibitory effects depends on the type of the medium and nanoparticles, but the fungal isolate had no significant effects on nanoparticles' inhibitory features.

Conclusions: Ag/Zn nanoparticles had no regular effect on the reduction of aflatoxin production, yet Ag nanoparticles reduced the production of aflatoxins, especially aflatoxins B2 and G2.

Keywords: Antifungal; Inhibitory; Nanoparticle; Silver; Zinc

1. Introduction

Mycotoxins are secondary metabolites produced by certain filamentous fungi [1-2, 3]. Over 20 species of *Aspergillus* produce mycotoxins; however, the major producers of aflatoxins are those of the species *Flavi*, including *A. flavus*, *A. parasiticus*, and *A. nomius.* Four types of naturally-produced aflatoxins are B_1, B_2, G_1, and G_2. B_1 *Aspergillus parasiticus* commonly infects cereal grains and peanuts, and as a result produces aflatoxins. Aflatoxin is one of the carcinogenic, mutagenic and teratogenic substances extracted naturally from foods and feeds [4-5]. The consumption of mycotoxin-contaminated foods or crops has been linked with several cases of human and animal poisoning or mycotoxicosis, sometimes resulting in death [6]. Storage fungi are commonly controlled by synthetic chemicals, yet most of fungicides cause some side effects [7-8]. Consumers usually demand foods without preservatives and mycotoxins. [9]. Nowadays, the importance of alternative indigenous products to control phytopathologenic and mycotoxigenic fungi is becoming prevalent [10-11]. Efforts to provide alternatives to pesticides have been persistently made, since the overuse of pesticides causes ecological and environmental problems, and exerts harmful effects on humans. Nanotechnology is an enabling state-of-the-art technology that deals with structures ranging from approximately 1 to100 nm in at least one dimension [12]. The nanosize results in specific physicochemical features that may differ from those of the bulk substances or particles of larger sizes. This feature is mainly attributed to the high surface area to the volume ratio that potentially results in high reactivity. Due to such specific characteristics, the use of nanoparticles may have advantages over the use of bulk chemicals. Nanotechnology is developing rapidly and is used in various fields, including health care, consumer products like cosmetics, foods, feeds, and agricultural products [13]. Silver (Ag) is known as a powerful nanomedicine used as a disinfecting agent for killing microorganisms by inactivating their metabolically enzymes [14]. Silver ions are very reactive and lead to the inhibition of microbial respiration and metabolism [15-16]. Moreover, it has been suggested that silver ions intercalate into the bacterial genome upon entering the cell and prevent the further replication and propagation of the pathogens [17]. Nanotechnology has recently improved the effectiveness of silver particles as antimicrobial agents. The

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larger surface area-to-volume ratio of silver nanoparticles increases their contact with microbes and their ability to permeate cells. Nanoparticle development has restored the interest in the antimicrobial effects of metals that had declined following the widespread application of modern synthetic antibiotics. Unfortunately, research on the antimicrobial effects of silver nanoparticles has been conducted mostly on animal pathogens [15-17-19, 18]. The objective of this study is to develop a safe method to control *A. parasiticus* in cases of mycelial growth, spore germination and aflatoxin production, using Ag/Zn and Ag nanoparticles.

2. Materials and Methods

2.1. The synthesis of Ag/Zn nanoparticles

Ag/Zn nanoparticles were prepared as follows; two 1 M solutions of Zn (AC) 2.2H2O and AgNO3 in EtOH/H2O were prepared. A 10 M sodium hydroxide solution was added to the above mixture at room temperature under stirring. The final solution prepared was stirred for 60 min, and it was then transferred into Teflon-lined stainless steel autoclaves, then sealed, and heated at 155°C for 12 h. Subsequently, the reactor temperature was immediately lowered to -15°C. The particles prepared were extracted from the solution through centrifuging them, and washing them by distilled water and ethanol, and they were finally dried at 50°C in air.

2.2. The synthesis of Ag nanoparticles (NPs)

Ag nanoparticles were prepared using a solution of silver nitrate containing 24–30% (by weight) of silver nitrate and 28–34% of ammonium hydroxide, and a solution used as an activator containing 7–12% of sodium hydroxide and 6–10% of ammonium hydroxide, and finally a reducer solution containing 0.3% of formaldehyde and 27–33% of sorbitol.

0.5 ml of the prepared silver solution was added to 14 ml of the deionized water, and then 0.5 ml of the activator solution was added to it. The solution prepared was then diluted to 300 ml, and it was referred to as "SAB". 0.5 ml of the reducer solution was diluted to 300 ml, and it was referred to as "SC". Silver nanoparticles were prepared by adding 0.9 ml of SAB to 10 ml of SC solutions and were located at a sonication bath from several minutes to half an hour.

2.3. Fungal isolates identification

Aspergillus parasiticus (isolates no. 86 and 128) was identified according to the morphological features and phylogenetic analysis of the partial calmodulin gene sequence, obtained from *Aspergillus* culture collection, the department of plant protection, Vali-e-Asr university of Rafsanjan, Iran. The fungi were originally isolated from pistachio nuts. The ability of the aflatoxin production of fungal isolates was tested on the yeast extract sucrose agar medium using the TlC method [20].

2.4. Effects of nanoparticles on mycelia growth in solid medium

A solution of 25, 50, 100, 200, 500, 750, 1000, and 1250 ppm of Ag-Zn, and a solution of 25, 50, 100, and 200 ppm of Ag nanoparticles were prepared in Potato dextrose agar (PDA, Merck, Germany) and Czapeck Dox agar (CZ, Merck, Germany). The nanoparticles were added to the medium after sterilization, just before being poured to the plates (~ 40 °C). 20 ml of the medium were poured to 10 cm of Petri dishes. A small disc of the fresh colony was placed at the center of the plates. The plates were incubated for 7 min at 25°C, in the dark. The experiment was designed according to the randomized blocks. All experiments were replicated three times. The colony diameter of treatments (t) was compared with the control one (c). The growth rates of the vegetative mycelia were measured according to the following equation [21]:

$$\text{Inhibitory rate} = \frac{\text{Avg mycelia}[t] - \text{Avg mycelia}[c]}{\text{Avg mycelia}[c]}$$

2.5. Effects of nanoparticles on mycelia growth in the liquid medium

Potato dextrose broth (PDB) and CZ liquid media were autoclaved, and 100 ml of each were distributed in 250 ml of Erlenmeyer. The mentioned concentrations of Ag-Zn and Ag nanoparticles were added to the medium and inoculated with four 5 mm disks of the fresh colony of the fungal isolates; they were then incubated in a shaker incubator at 150 rpm at 25ºC, for 7 days. The mycelia were harvested and washed by a Buchner funnel and a vacuum pump. The mycelia's dry weight was measured after a 72 h drying process at 48 ºC. All experiments were replicated three times.

2.6. Spore germination assay

The spore suspension of *A. parasiticus* was prepared at the mentioned concentrations of Ag-Zn and in the Ag nanoparticles solution, and it was shacked at 150 rpm for 7 days at 25°C. The 0.5 ml of 5×10^6 spore/ml suspension was cultured on PDA. Sterile distilled water was used as the control agent. The percentage of the germinated spores was counted under an optical microscope after 24 h [21].

2.7. Fungal Cultivation and Aflatoxin Production

The aflatoxin produced by *A. parasiticus* was tested on the yeast extract sucrose (YES) medium. 50 ml of the medium were poured in each 250 ml Erlenmeyer flask. The flasks were sterilized by an autoclave at 121ºC for 15 minutes, and at the mentioned concentration of Ag and Ag/Zn, the nanoparticles were added to flasks. Inoculation was carried out by adding 1 ml of the spore suspension (10^5 spores) of *A.*

parasiticus, and the flasks were incubated at 28-30°C for 14 days.

2.8. Toxin extraction

After 14 days, 4 ml of chloroform were added to each Erlenmeyer and shacked thoroughly. After 30 minutes, the product was mixed again and 1 ml of the chloroform phase (the lower phase) was transferred to the test tube and centrifuged for 10-15 minutes at 3000 rpm. In order to determine the concentration of the toxins, the lower phase was transferred to micro-tube and placed under the chemical hood at 70 ^{o}C for 30 minutes.

2.9. Toxin analysis

2.9.1. Reagent

Aflatoxins of B_1, B_2, G_1, and G_2 standards were procured from Sigma (Mo, USA). Methanol, acetonitrile, potassium bromide, nitric acid (Merck, Darmstadet, Germany), and water of the high-performance liquid chromatography (HPLC) grade were used in the present research.

2.9.2. Standard solutions

After the preparation of the standard solutions for each aflatoxin, their concentrations were determined using the UV-visible spectrophotometer (Varian, Cary 100, USA) through the AOAC official method no.971.22 (AOAC, 2006; chap. 49.2.03). These standards were used to prepare mixed standards for the HPLC analysis. The working standards were prepared by diluting the tertiary stock standard 40 ng/ml (AFB_1, AFG_1=16 ng/ml; AFB_2, AFG2= 4 ng/ml) at the methanol gradient grade.

2.9.3. Apparatus

Liquid chromatography (LC) analysis was performed using a reverse phase HPLC system (Dionex, Sunnyvale, California LP USA) equipped with a Gilson workstation (GX-271 ASPEC Gilson, USA), a vacuum degasser (Ultimate-3000, Dionex , Sunnyvale, California LP, USA), a temperature-controlled oven (Ultimate-3000, Dionex, Sunnyvale, California LP, USA), and a fluorescent detector (RF2000, Dionex , Sunnyvale, California LP, USA). The dionex LC column was C_{18}, 250×4.6 mm, 5µm. Afla test immunoaffinity columns (IAC) were purchased from VICAM (MA, USA).

2.9.4. Chromatographic conditions

The reversed-phase LC determination of aflatoxins was conducted using the post-column bromination with Kobracell (Coring system, Gernsheim, Germany) at a flow rate of 0.8 ml/min, the fluorescence detection at the excitation wavelength of 365 nm and the emission wavelength of 435 nm. The column temperature was adjusted at 36 °C. The retention times for AFG_2, AFG_1, AFB_2, and AFB_1 were 10.50, 12.07, 13.56, and 16.13 min, respectively. The isocratic mobile phase was a water-acetonitrile-methanol solution in the ratio of 60:20:30 (v/v/v), containing 110 mg/L of KBr and 350 µL of HNO_3 4M.

2.9.5. Extraction and cleanup

The samples were analyzed for the aflatoxin content, based on AOAC, 2006; chap. 49.2.29 with minor modifications. The extraction procedure was followed by adding 1 ml of methanol/water 80/20 (v/v), and the resulting solvent collected into a 20 ml flask was shacked for 1min. The procedure was repeated three times, and the container was then washed by the solvent to reach 20 ml (one mL of the extract diluted with 10 mL of the solvent, two times), and he extract was filtered through MN619de filter paper. From the filtered extract, ca. 10 ml in a tube was placed in ASPEC (Gilson GX271), and 3.1 ml of the extract was diluted with 9.9 ml of deionized water and then mixed with 8 ml of air. To clean up the diluted extract, Afla test immunoaffinity columns (IAC) were used. 12.6 ml of the diluted extract were filtrated through the IAC using the workstation (ASPEC) cleaned needle at the flow rate of ca.1 drop/s. The column was washed using 15 ml of water and dried through passing 2 ml of air. Next, aflatoxin was eluted with 1.5 ml of methanol at the flow rate of ca.1 drop/2s. Finally, the remaining methanol was collected through passing 1.5 ml of air. The eluted aflatoxin was diluted with 1.5 ml of deionized water, after which 100µl of eluted solution was injected into HPLC.

2.9.6. Calibration curve

A calibration curve was prepared using the working standard solutions. The tertiary stock standard was used to prepare the working standard solutions through pipetting appropriate volumes into a set of 20 ml of calibrated volumetric flasks and diluting the volumes. The concentrations of AFB_1 and AFG_1 were 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 2.4 ng/ml, and the concentrations of AFB_2 and AFG_2 were 0.00625, 0.0125, 0.025, 0.05, 0, 1, 0.2, 0.4, and 0.6 ng/ml. An eight-point calibration curve was designed for each type of the aflatoxins. The calibration curve was prepared before the analysis to check the pilot for linearity (the coeff.det percentages for AFB_1, AFB_2, AFG_1 and AFG_2 were 100.00, 99.982, 99.999, and 99.999, respectively), and it was used to quantify aflatoxins.

2.9.7. Quality assurance

To evaluate the reliability of the results, in addition to using validated methods, the internal and external quality control experiments were performed. Regarding the internal quality control, the accuracy and precision of the methods were verified. For this purpose, AFB_1, AFB_2, AFG_1, and AFG_2 were recovered through analyzing a blank pistachio slurry sample and a spiked one at 4 ng/g for AFB_1 and AFG_1, and

at 1 ng/g for AFB_2 and AFG_2. Aflatoxin levels were corrected according to the recovery values. The results were quantified to show the amount of aflatoxins in each container, so the results are presented in ng. Regarding the external quality control, Rafsanjan Food Control Lab (RFCL) participated regularly in the proficiency testing for the Food Analysis Performance Assessment Scheme (FAPAS) in UK and obtained a consistently satisfactory Z-score ($-2 \leq$ Z-score $\leq +2$).

2.10. Data analysis

The experiments were designed with a fully randomized design, and the data were analyzed statistically using the software SPSS (Inc ver. 16), with the analysis of variance (ANOVA) and the multiple comparison at a 0.5% level.

3. Results

3.1. Structural Study

The morphology, structure, and size of the samples were assessed using the TEM analysis. Particles have round shapes (Fig. 1. (a)), with most of them having a diameter of about 20 nm (Fig. 1. (b).

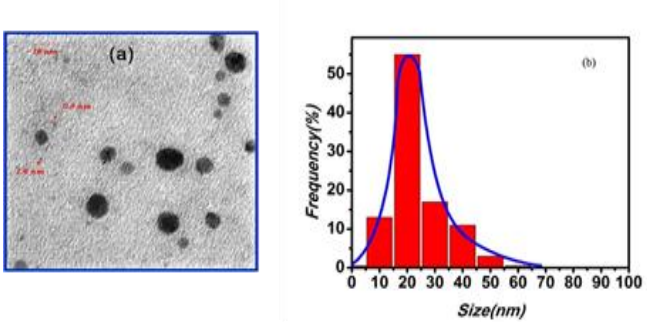

Fig.1. TEM image (a) and size distribution histogram (b) of synthesized Ag NPs

The XRD pattern of the Ag-synthesized NPs is shown in Fig. 2.

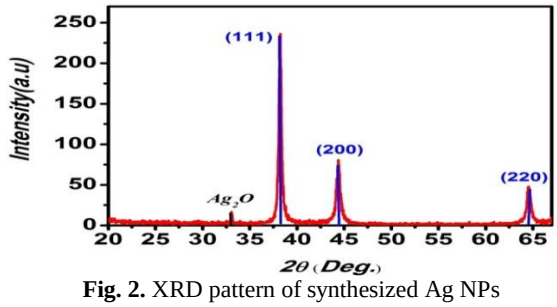

Fig. 2. XRD pattern of synthesized Ag NPs

The positions of the three main peaks correspond to (111), (200), and (220) planes of the cubic FCC structure. The broadening of diffraction peaks indicates the formation of NPs. Using the full width at half maximum (FWHM) of the first main XRD peak and Debye–Scherrer's formula, the crystallite sizes of Ag NPs are estimated at 29 nm. Fig. 3 shows the absorption spectra of the synthesized NPs. The Plasmon peak of silver is clearly observable at about 420 nm.

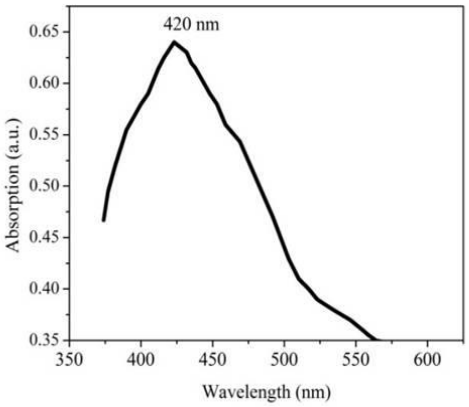

Fig. 3. Absorption spectra of synthesized silver NPs.

3.2. Mycelia growth on solid medium

The inhibitory rate of the mycelia growth depends on the type and concentration of nanoparticles. The fungal isolate colony diameter in both PDA and CZ media contains a concentration higher than 200 and 50 ppm of Ag-Zn and Ag nanoparticles, respectively, being significantly lower than the concentration of the control agent. The inhibitory rates of mycelial growth at the concentrations of 500, 750, 1000 and 1250 ppm of Ag/Zn nanoparticles in PDA and CZ media were 36, 36, 44, 60% and 15, 22, 36, and 54%, respectively. The concentrations of 100 and 200 ppm of Ag nanoparticles had 20 and 34% inhibitory effects in PDA and 23 and 41% in CZ medium. The effects of the nanoparticles are almost the same on both fungal isolates. The medium type had no significant effect on the inhibitory rate of the mycelial growth (Tables 1, 3 and Fig. 4).

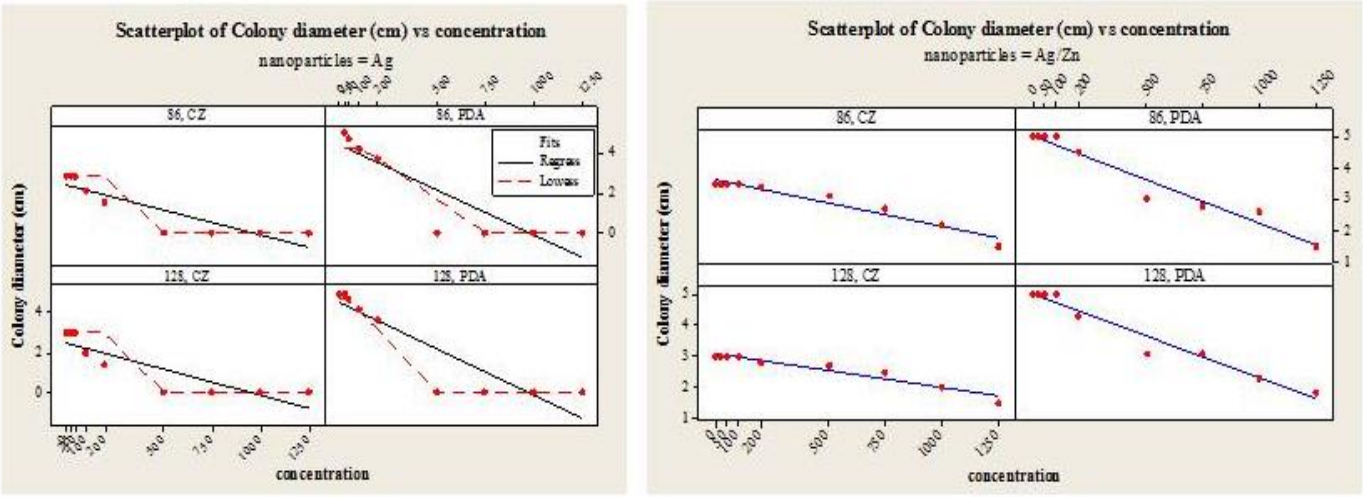

Fig. 4. The regression of colony diameter under nanoparticles concentration in PDA and CZ media.

Table 1. Variance analysis of the effect of nanoparticles concentration on colony diameter of *Aspergillus parasiticus* isolates in PDA and CZ media.

Source	DF	Colony diameter (cm)
Nanoparticles	1	38.39**
Strain	1	0.002^{ns}
culture	1	18.51**
concentration	8	118.71**
Error	59	33.03

3.3. Mycelia growth in the liquid medium

The mycelia dry weight indicated that upon an increase in the nanoparticle concentration, the mycelium dry weight decreased. Like the solid medium, the inhibitory rate of the nanoparticles was approximately the same according to fungal isolates, but the type of medium was effective on the inhibitory rate. In both media, the concentration of 25 ppm of Ag/Zn and Ag had no inhibitory effect. The concentrations of 50 and 100 ppm of Ag/Zn nanoparticles had no inhibitory effects on both fungal isolates in PDB, but they had 7% and 14% effects on CZ medium, respectively. The concentrations of 1250, 1000, 750, and 500 ppm of Ag/Zn had 100,100,100, and100 and 64, 38, 25, and 17% inhibitory effects on CZ and PDB media, respectively. However, the concentrations of 25 and 50 ppm of Ag nanoparticles had no significant inhibitory effects on both media as against fungal isolates. The concentrations of 100 and 250 ppm of Ag nanoparticles had about 57 and 92% of the inhibitory effect of both fungal isolates in both media examined (Tables 2, 3 and Fig. 5).

3.4. Spore germination

The inhibitory effect of nanoparticles on spore germination was significant. The results showed that after 24 h the surface of blank Petri dish was covered by germ tubes, and that the colonies were uncountable. In all concentrations of Ag/Zn and Ag nanoparticles, germinated spores were by far lower compared with the control plates (Fig. 6). The results showed that upon an increase in the concentration of the nanoparticles, the germinated spore percentage decreased.

3.5. The effects of Ag/Zn nanoparticles on the synthesis of aflatoxins

The results showed that not only Ag /Zn nanoparticles had no inhibitory effects on the synthesis of total aflatoxins, but they also increased B1, B2, and total aflatoxins, yet they reduced the production of aflatoxins B2 and G2 significantly. The concentrations of 500, 750, 1000 and 1250 ppm caused an 88, 81, 81, and 82% reduction in the aflatoxin B2 synthesis, respectively. Regarding aflatoxin G2, the same concentrations had the inhibitory effects of 3, 23, 31 and 42%, respectively (Table 4).

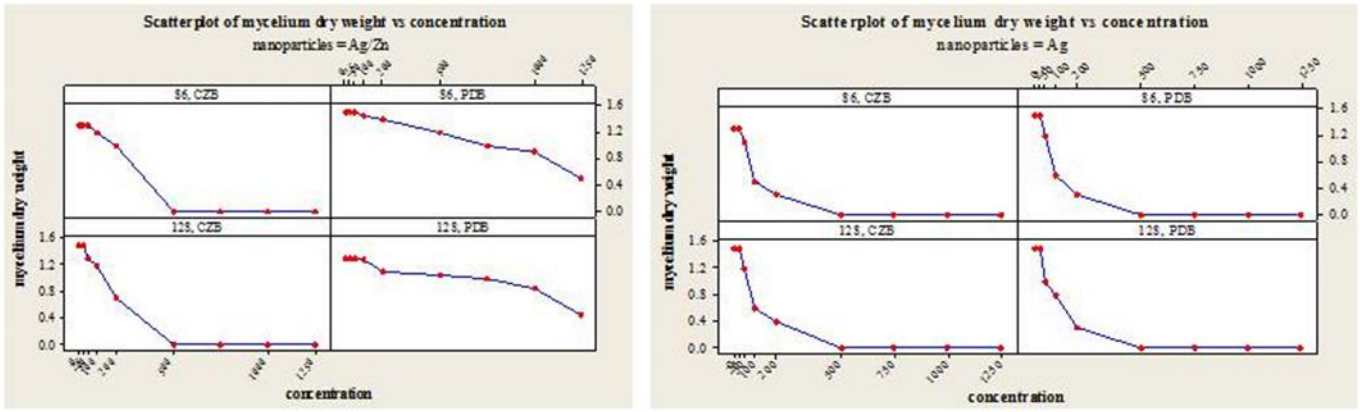

Fig. 5. The regression of mycelial dry weight under nanoparticles concentration in PDB and CZB media.

Table 2. Variance analysis of the effect of nanoparticles concentration on mycelial dry weight of *Aspergillus parasiticus* isolates in PDB and CZB media.

Source	DF	Mycelium dry weight (g)
Nanoparticles	1	2.33**
Strain	1	0.003^{ns}
culture	1	1.06**
concentration	8	18.71**
Error	60	4.04

3.6. The effects of Ag nanoparticles on the synthesis of aflatoxins

As against Ag/Zn nanoparticles, Ag nanoparticles caused a moderate reduction in the total synthesis of aflatoxins. Upon the concentration increase to 100 ppm, the inhibitory effects increased, but the concentrations 100 and 200 ppm had the same results. Similar to Ag/Zn nanoparticles, aflatoxins B2 and G2 showed more reduction in case of being treated by Ag nanoparticles. For the concentrations of 25, 50, and 100 ppm, the reduction rates were 45, 47, 57% and 88, 91, 94 % for aflatoxins B2 and G2, respectively (Table 5).

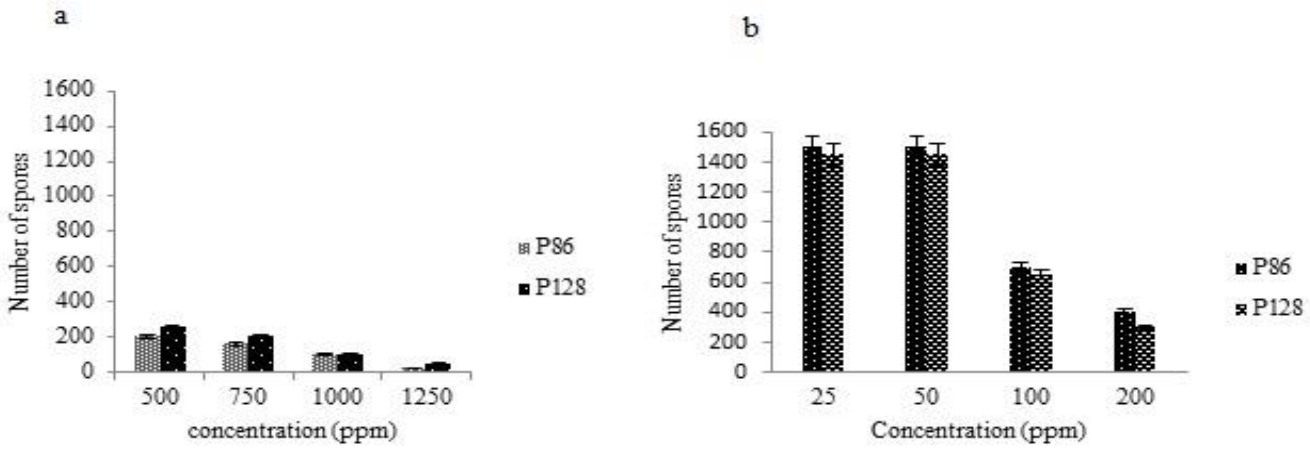

Fig. 6. The effect of nanoparticles on spore germination, Silver-zinc (a) and Silver (b)

Table 3. The effect of Ag/Zn and Ag nanoparticles on colony diameter and mycelium dry weight of *Aspergillus parasiticus* strains.

	Colony diameter (cm)								Mycelium dry weight (g)							
Nano concentration (ppm)	Ag/Zn		Ag/Zn		Ag		Ag		Ag/Zn		Ag/Zn		Ag		Ag	
	86		128		86		128		86		128		86		128	
	CZ	PDA	CZ	PDA	CZ	PDA	CZ	PDA	CZB	PDB	CZB	PDB	CZB	PDB	CZB	PDB
25	3.5	5	3	5	2.8	5	3	5	1.3	1.5	1.5	1.3	1.3	1.5	1.5	1.5
50	3.5	5	3	5	2.8	4.7	3	4.7	1.3	1.5	1.3	1.3	1.1	1.2	1.2	1
100	3.5	5	3	5	2.1	4.2	2	4.2	1.2	1.45	1.2	1.28	0.5	0.6	0.6	0.8
200	3.4	4.5	2.8	4.3	1.5	3.7	1.4	3.7	1	1.4	0.7	1.1	0.3	0.3	0.4	0.3
500	3.1	3	2.7	3.1	-	-	-	-	0	1.2	0	1.05	-	-	-	-
750	2.7	2.8	2.5	3.1	-	-	-	-	0	1	0	0.99	-	-	-	-
1000	2.2	2.6	2	2.3	-	-	-	-	0	0.9	0	0.85	-	-	-	-
1250	1.5	1.5	1.5	1.8	-	-	-	-	0	0.5	0	0.45	-	-	-	-
C	3.5	5	3	5	2.8		3	5	1.3	1.5	1.5	1.3	1.3	1.5	1.5	1.5

Table 4. The effect of Ag/Zn nanoparticles concentration on aflatoxin production by *Aspergillus parasiticus*

Nano concentration (ppm)	B1	B2	G1	G2	TOTAL
C	323.4	190.53	36.16	4.04	554.13
500	536.5	21.98	100.77	3.89	663.14
750	573.2	35.23	319.51	3.105	931.045
1000	449.9	35.39	337.43	2.78	825.5
1250	447.3	33.51	320.20	2.31	803.32

Table 5. The effect of Ag nanoparticles concentration on aflatoxin synthesis by *Aspergillus parasiticus*

Nano concentration (ppm)	B1	B2	G1	G2	TOTAL
C	127.88	39.80	87.88	7.13	262.69
25	125.11	21.5	85	0.81	232.42
50	123.3	20.7	80.8	0.6	225.4
100	120.1	17	70	0.4	207.5
200	120.1	17	70	0.3	207.4

4. Discussion

Managing fungal diseases especially mycotoxigenic phytopathogenes on food crops is economically important. More efforts have recently been directed to developing safe management methods and means, including natural and biological substances as well as nanoparticles that pose less danger to the environment, humans and animals, and have been focused on overcoming the problems concerned with fungicides. Silver is a non-toxic, safe, inorganic and antimicrobial element that has been used to control many microbes [22]. The current experiment showed that silver-zinc and Ag nanoparticles were also quite effective in reducing the mycelial growth of *A. parasiticus*. However, the pollution rates of these findings compared with more general cases are not too high due to the fact that the current study is based on *in vitro* petri dishes. The current study suggests the possible use of Ag/Zn and Ag nanoparticles as an alternative to chemical pesticides in some crops, for instance, to eradicate phytopathogens and saprobe fungi, although there are some parameters to be evaluated for the practical use. This may involve the evaluation of phytotoxicity and antimicrobial effects in hosts and the development of the delivery system of silver-zinc and Ag nanoparticles in host tissues. The nanosized silver-zinc and Ag particles of the present study exhibit a wide range of activities to control spore germination, hyphal development and aflatoxin production. As many studies have focused on the bacterial disease control, little is known about the effects of silver particles on plant pathogenic fungi. The findings of the current study clearly demonstrated that silver nanoparticles inhibited fungal mycelial growth and spore germination significantly. Jo *et al.* [23] examined the effects of silver nanoparticles at the concentration of 500 ppm and observed the inhibition of mycelial growth of *Biopolaris sorokiana* and *Magnaporthe grisea in vitro*. Kim *et al.* [24] examined the concentrations of various forms of silver nanoparticles on the fungal hyphae and spore germination of the oak pathogen *Raffaelea* sp and confirmed a strong fungi static effect. Ionic silver nanoparticles inhibit the metabolism of microorganisms [25-26]. Amro *et al.* [27] suggested that metal depletion may lead to the formation of irregularly shaped pits in the membrane and change membrane permeability as a result of the progressive release of lipopolysaccharide molecules and membrane proteins. Nano-Ag breaks through the membrane permeability barrier of *Candida albicans* [28].

Other research findings suggest that the action mode of Ag nanoparticles is the same as that of silver ions that band with electron donor groups containing sulfur, oxygen or nitrogen atoms, being normally present as thiols or phosphates [29] in amino acids and nucleic acids. Like silver nanoparticles, silver ions do their activities by a range of mechanisms, including denaturing the 30s ribosome subunit, suppressing the expression of enzymes and proteins essential for ATP production [30], inhibiting respiratory enzymes, thereby inducing the production of reactive oxygen species [31-30], binding and dimerizing RNA and DNA [32], and destabilizing and disrupting the outer membrane [33].

Reports on silver ion-resistant strains have also indicated a modification in the response to antibiotics, namely developing resistance to mercuric chloride, ampicillin, chloramphenicol, tetracycline, streptomycin, and sulfonamides [34].

Ag and Ag/Zn nanoparticles do not exert significant inhibitory effects on any aflatoxin. That may be due to the fact that the element Zink helps stimulate aflatoxin production, and that Zn nanoparticles have higher stimulatory effects than Zink per se [35-36, 37]. The inhibitory effects of the element Ag on aflatoxin production were discussed by Marsh *et al.*, [38] and the present study

also showed that Ag nanoparticles could have inhibitory effects on aflatoxin production, yet not so strong ones. This study clearly demonstrates that Ag/Zn and Ag nanoparticles are powerful antifungal agents against *Aspergillus parasiticus* in the fields of agriculture and medicine.

5. Conclusions

The findings of the current study indicate that the application of Ag and Ag/Zn nanoparticles may be a useful treatment for agricultural wastes such as pistachio hulls that are the main source of primary inoculums and overwintering of mycotoxigenic fungi. Moreover, since these residues are currently processed and utilized in organic fertilizers, some of such nanoparticles can be used to enrich fertilizers and concurrently reduce the population of toxigenic fungi.

Conflicts of interest

The authors declare no conflicts of interest.

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