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Original Article

Inhibitory Effects of Silver Nanoparticles on Growth and Aflatoxin B_1 Production by *Aspergillus Parasiticus*

Seyyed Amin Ayatollahi Mousavi[1], PhD;
Somayyeh Pourtalebi[2], MSc

Abstract

Background: Aflatoxins (AFs) are secondary hazardous fungal metabolites that are produced by strains of some *Aspergillus* species on food and feedstuffs. Aflatoxin B_1 (AFB_1) is one of the most important AF with high toxicity. Prevention of AF production and their elimination from food products is a matter of importance for many researchers in the last decades. Nanomaterials applications in medical science have been widely studied in the recent years. Most of existing researches seek the effect of nanoparticles on bacteria, fungi, and viruses. The aim of this study was to determine the effects of silver nanoparticles (AgNPs) on growth and AFB_1 production of AF-producing *Aspergillus parasiticus*.

Methods: *A parasiticus* was inoculated (10^6 conidia per ml of medium) to potato dextrose broth (PDB) medium and then AgNPs was added and incubated with shaking at 130 rpm and 28°C for 7 days. AF was assayed by high performance liquid chromatography (HPLC). Microbiological assay (MBA) on microplates contained potato dextrose broth (PDB) medium (4 days at 28°C) at different concentrations of AgNPs (60, 80, 100, 120, 140, 160, 180 and 200 µg/ml) was measured.

Results: The results demonstrated that a minimum inhibition concentration (MIC) equal to 180 µg/ml was determined for AgNPs against *A. parasiticus*. The AgNPs effectively inhibited AFB_1 production at a concentration of 90 µg/ml.

Conclusion: The results obtained in this study show AgNPs at concentrations lower than the MIC drastically inhibited production of AFB_1 by *A. parasiticus* in culture medium. The AgNPs may be useful to control AF contamination of susceptible crops in the field.

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[1]Department of Medical Mycology and Parasitology, School of Medicine, Kerman University of Medical Science, Kerman, Iran;
[2]Department of Microbiology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

Correspondence:
Somayeh Pourtalebi, MSc;
Department of Microbiology, Persian Gulf Blvd, Pistachio Blvd, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
Tel: +98 34 34339042
Fax: +98 34 34339660
Email: somayeh.pourtalebi@yahoo.com
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Introduction

Aflatoxins (AFs) are secondary metabolites with toxic and carcinogenic effects, which are produced by species of Aspergillus, particularly Aspergillus flavus and Aspergillus parasiticus (*A. parasiticus*).[1] The commodities such as peanuts, rice, corn and cottonseed have suitable condition for growth of these fungi, so contamination of these commodities with AFs often makes them unfit for consumption.[2,3]

AFs are considered the most carcinogenic, mutagenic, and teratogenic compounds found naturally in foods and feeds.[4] Aflatoxins B_1 (AFB_1), B_2 (AFB2), G_1 (AFG_1), and G_2 (AFG_2) are major naturally occurring AFs produced by aflatoxigenic fungi.[5] AFB_1 is the most powerful AF; moreover, AFB_1 has been classified as a human carcinogen in the group 1 by the International Agency for Research on Cancer (IARC).[6,7] In addition, AFB_1 is a hepatocarcinogenic compound that might cause tumors in other organs like colon and kidney.[8] The importance of AFB_1 led to many researches on the effects of different compounds in order to inhibit its production.[9] Nanotechnology for the purpose of manufacturing new materials at nanoscale is considered as rapidly developing field that a large spectrum of research has been focused on its application.[10,11] Different types of nanomaterials like copper, zinc, platinum, titanium, magnesium, gold, alginate and silver have come up, but silver nanoparticles (AgNPs) have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms. For many years, silver has been in use to treat burns and chronic wounds.[11] Also silver and its compounds are regarded as a relatively safe antimicrobial metal that has a wide spectrum of activity.[12,13]

Recently, due to enhancement of antibiotic-resistant bacteria and limitations of antibiotic usage, silver wound dressings with a variety of silver levels is used again as an alternative treatment.[14] Due to new advances of researches about metal nanoparticles, nano-Ag has received special attention as a possible antimicrobial agent, which in low concentrations is nontoxic.[15,16] AgNPs have higher effective antimicrobial activity compared to bulk silver metal.[17]

Nanoparticles due to their size have a larger surface area to come in contact with microorganisms than a bigger form of particles. Therefore, nanoparticles have a higher percentage of interaction.[11,18,19]

Antifungal activities of AgNPs against different kinds of fungus have not been reported as much as antimicrobial activities of them by researchers.[16] It was reported that AgNPs could inhibit fungi in low concentrations and those levels had no toxic effect on human cells.[20] In a research by Kim et al., it is indicated that AgNPs have antifungal activity against Trichophyton mentagrophytes, Trichosporon beigelii, and Candida albicans in comparison with available antifungal agents like amphotericin B and fluconazole.[15,21]

The aim of this study was to investigate the effects of AgNPs on the growth and AFB_1 production by *A. parasiticus*.

Materials and Methods

Fungal Strain, Growth Media and Chemicals

The *A. parasiticus* PTCC 5280, a known producer of AFB_1 was used throughout the study. Potato Dextrose Broth (Merck, Germany) was the culture medium used for AFs production by the fungus.[22] AgNPs in colloid form with 4,000 ppm were purchased from Nanocid Company in Tehran (www.Nanocid.com).

Minimal Inhibitory Concentration

A. parasiticus was cultured on Potato Dextrose Broth in 10-well flat-bottom microplates (IWAKI; well dia. 16 mm). The culture medium was added to microplates in amounts of 1 ml/well and then inoculated with fungal spore suspension (1×10^6 spores/well) prepared in sterilized distilled water containing 0.1% Tween 80. Different concentrations of the AgNPs (60, 80, 100, 120, 140, 160, 180 and 200 ppb) prepared in dimethyl sulfoxide (DMSO) were added to the test wells.[23] Ketoconazole and sterile distilled water (SDW) were used as positive control and negative control, respectively. The microplates were incubated for 96 h at 28°C in 130 rpm. Growth of mold was observed visually throughout the incubation period. The minimal inhibitory concentration (MIC) was defined as the concentration of AgNPs that prevented growth in the media as determined visually.

Effect of Silver Nanoparticles on Aflatoxin

For these experiments 250 µl of a suspension containing 1×10^6 spores/ml were added to 250 ml Erlenmeyer flasks containing 50 ml of broth in the presence of AgNPs at concentrations lower than the MIC (75%, 50%, and 25% MIC). Following the addition of each solvent, the cultures were shake-agitated at 130 rpm for 7 days.

Determination of Aflatoxins Production

A 50 ml of Potato Dextrose Broth was mixed with 2.5 g NaCl and 100 ml solution of 80% methanol in water for 5 min and then the mixture was filtered on Whatman filter paper. The extract was diluted with 25 ml of distilled water and 5 ml PBS with pH 7.4. Finally, 20 ml of this was purified on the immunoaffinity column activated with 20 ml of PBS by gentle syringe pressure at a flow rate of 1 ml/min and then the column was washed with 20 ml of deionized water. The column was dried by blowing air through it for 2–3 seconds with a syringe. The AFs were slowly eluted from the column with 2 ml of HPLC

grade methanol and then were diluted with 2 mL of deionized water.[24] A 50 µl sample loop was used to inject the sample into the HPLC.

Results

Antifungal Activities of Silver Nanoparticles on A. Parasiticus

Initial data showed that AgNPs was able to inhibit *A. parasiticus* growth and AF production effectively. Antifungal activity was confirmed for AgNPs (data not shown in details).

Inhibition of A. Parasiticus Growth by AgNPs

The AgNPs was effectively inhibited fungal growth in microbioassay at some concentrations used. This inhibition was reported in concentration of 180 µg/ml.

Inhibitory Effects on Aflatoxin Production

Figure 1 illustrates AFB_1 production without the effect of AgNPs, where the determined point was the start time of AF production. The HPLC results of AF production showed that these nanoparticles were able to strongly inhibit AFB_1 production in a dose dependent manner, also AFB_1 inhibition was reported at a concentration of 90 µg/ml after comparing with non-treated controls (Figure 2).

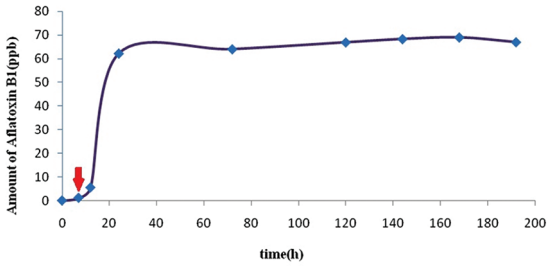

Figure 1: Shows Aflatoxin B_1 production by *A. Parsiticus* without silver nanoparticle effect. Arrow: Start time of Aflatoxin production (7 h).

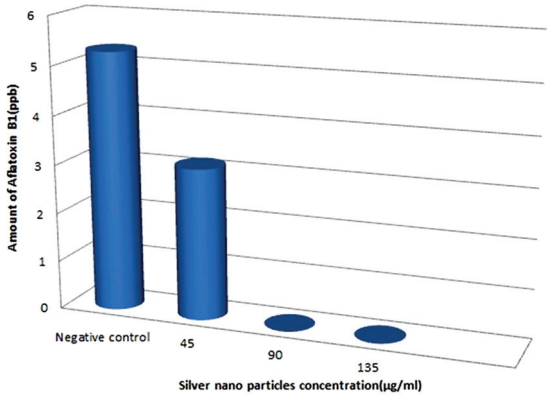

Figure 2: Shows different silver nanoparticles concentrations effect on Aflatoxin B_1 production, at 90 µg/ml (50% MIC) AgNPs, Aflatoxin production was inhibited.

Discussion

Silver is one of the most effective antibiotic substances known and has been used to treat human ailments for over 100 years due to its natural antibacterial and nontoxic properties.[25] Since nano-sized silver materials have stronger antifungal activity than bulk silver materials, it has recently attracted great attention.[20] In fact, smaller sized particles possess a larger surface area in contact with the microorganism and have a higher percentage of interaction than bigger particles. Hence, AgNPs antibacterial activity is more efficient. AgNPs has found a variety of applications in different fields, especially medical science.[26]

Researchers reported the antifungal activity of AgNPs on the same fungus species.[20] Although most of the studies are focused on nanoparticle applications, there are limited studies describing the impact of nanoparticles on human health.[27,28] Results of last researches showed that MIC of AgNPs was lower than the cytotoxicity level of tested human cells. Therefore, low concentrations of silver nanoparticles could inhibit fungi and those levels had no toxic effect on human cells.[20]

Panacek et al. have claimed that nano-sized silver has a significant antifungal activity against *Candida albicans*.[29] Also, in another study by Kim et al., Antifungal effects of AgNPs on *Candida albicans* were investigated.[15,21] The MIC values of these studies indicated that AgNPs inhibited growth of *Candida albicans* at low concentrations (<4 µg/ml). In comparison with the last two studies, the present research shows that *A. parasiticus* growth is inhibited at higher concentration of AgNPs. This discrepancy can be derived from different species of fungi and AgNPs used in both works, where AgNPs were stabilized by surfactants and polymers.

In our study, minimum inhibition concentration was determined by microdilution method. This method was similar to the method performed by Nozari et al., who reported that AgNPs showed an antifungal effect against *Candida* species isolated from chronic candida vulvovaginitis.[30]

Many studies have shown the antimicrobial effects of AgNPs.[13,31] Among the antimicrobial activity of AgNPs against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* was investigated by Kim et al. The MIC of AgNPs against *S. aureus* and *E. coli* was 100-150 µg/ml and in contrast with our study AgNPs were used in powder form.[32]

Antifungal effects of AgNPs on dermatophytes especially *Trichophyton mentagrophytes*, had been studied by Kim.[21] Some researchers have reported that the activity of antifungal drugs

against *Phoma glomerata, Phoma herbarum, Fusarium semitectum* and *Trichoderma sp* was enhanced in combination with AgNPs.[16,17] Therefore these studies confirm the antifungal effect of AgNPs on some fungi species. However, in spite of recent researches, the effects of nano-Ag against AF producing *A. parasiticus* and AFB_1 are mostly unknown.

Based on previous studies, the main contribution of this study was AgNPs effect on growth and AFB_1 production by *A. parasiticus*.

In the present study, we showed AgNPs inhibiting AFB_1 production by *A. parasiticus* in addition to the ability for strong fungal growth inhibition. Therefore the MIC value of the AgNPs against *A. parasiticus* was 180 µg/ml and AgNPs inhibited AFB_1 production at 50% of the MIC (90 µg/ml). When different concentrations of AgNPs were added to the fungal cultures in media, a remarkable inhibition in aflatoxin synthesis was observed. The inhibition in growth and toxin production was dependent on the concentration of AgNPs. In this study, since the AgNPs were used as antifungal drug, antifungal drug of Ketoconazole and sterile distilled water (SDW) were positive control and negative control, respectively. In a research by Gajbhiye et al., fluconazole, which is antifungal agent, was used as positive control for comparison with Ag-NPs[16] and Kim et al. used amphotericin B as a positive control in their study.[21]

To the best of our knowledge, this research was the first study about the effects of AgNPs on AFB_1 production by *A. parasiticus*. Due to the important role of AF contamination, especially negative effect of AFB_1 on public health, efforts should be conducted to prevent the contamination, since prevention is the most economical and practical approach. AgNPs ability indicates that they may be considered as useful candidates to eliminate AF contamination in food and feedstuffs.

Conclusion

Based on the results of the present study, AgNPs could inhibit growth and AFB_1 production by *A. parasiticus.* Therefore, it is concluded that nano-Ag has considerable antifungal activity. These nanoparticles must be subjected to further study to determine their effects on other mycotoxins, define toxicity, and evaluate economic feasibility.

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Conflict of Interest: The authors hereby declare that the prescribed silver nano particle in this study was prepared by Nanocid Company in Tehran.

References

1. Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Yoshinari T, Rezaee MB, Jaimand K, Nagasawa H, et al. Inhibitory effects of Satureja hortensis L. essential oil on growth and aflatoxin production by Aspergillus parasiticus. Int J Food Microbiol. 2008;123:228-33. doi: 10.1016/j.ijfoodmicro.2008.02.003. PubMed PMID: 18353477.
2. Moschini M, Gallo A, Piva G, Masoero F. The effects of rumen fluid on the in vitro aflatoxin binding capacity of different sequestering agents and in vivo release of the sequestered toxin. Anim Feed Sci Technol. 2008;147:292-309. doi: 10.1016/j.anifeedsci.2008.01.010.
3. El-Nagerabi SAF, Al-Bahry SN, Elshafie AE, AlHilali S. Effect of Hibiscus sabdariffa extract and Nigella sativa oil on the growth and aflatoxin B1 production of Aspergillus flavus and Aspergillus parasiticus strains. Food Control. 2012;25:59-63. doi: 10.1016/j.foodcont.2011.09.033.
4. Sanchez E, Heredia N, Garcia S. Inhibition of growth and mycotoxin production of Aspergillus flavus and Aspergillus parasiticus by extracts of Agave species. Int J Food Microbiol. 2005;98:271-9. doi: 10.1016/j.ijfoodmicro.2004.07.009. PubMed PMID: 15698688.
5. Yabe K, Chihaya N, Hatabayashi H, Kito M, Hoshino S, Zeng H, et al. Production of M-/GM-group aflatoxins catalyzed by the OrdA enzyme in aflatoxin biosynthesis. Fungal Genet Biol. 2012;49:744-54. doi: 10.1016/j.fgb.2012.06.011. PubMed PMID: 22750656.
6. Liu S, Qiu F, Kong W, Wei J, Xiao X, Yang M. Development and validation of an accurate and rapid LC-ESI-MS/MS method for the simultaneous quantification of aflatoxin B1, B2, G1 and G2 in lotus seeds. Food Control. 2013;29:156-61. doi: 10.1016/j.foodcont.2012.05.069.
7. Sidhu OP, Chandra H, Behl HM. Occurrence of aflatoxins in mahua (Madhuca indica Gmel.) seeds: synergistic effect of plant extracts on inhibition of Aspergillus flavus growth and aflatoxin production. Food Chem Toxicol. 2009;47:774-7. doi: 10.1016/j.fct.2009.01.001. PubMed PMID: 19167450.
8. Corcuera LA, Arbillaga L, Vettorazzi A,

Azqueta A, Lopez de Cerain A. Ochratoxin A reduces aflatoxin B1 induced DNA damage detected by the comet assay in Hep G2 cells. Food Chem Toxicol. 2011;49:2883-9. doi: 10.1016/j.fct.2011.07.029. PubMed PMID: 21855598.
9. Ghorbanian M, Razzaghi Abyabeh M, Allameh AA, Shams Ghahfarokhi M, Babaee GR, Rezaee MB. Effect of incubation time on Aspergillus parasiticus growth and its aflatoxin productivity in presence of neem. Hakim Research Journal. 2005;8:54-60. Persian.
10. El-Rafie MH, Shaheen TI, Mohamed AA, Hebeish A. Bio-synthesis and applications of silver nanoparticles onto cotton fabrics. Carbohydr Polym. 2012;90:915-20. doi: 10.1016/j.carbpol.2012.06.020. PubMed PMID: 22840020.
11. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 2009;27:76-83. doi:10.1016/j.biotechadv.2008.09.002. PubMed PMID: 18854209.
12. Klasen HJ. Historical review of the use of silver in the treatment of burns. I. Early uses. Burns. 2000;26:117-30. PubMed PMID: 10716354.
13. Silver S. Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS Microbiol Rev. 2003;27:341-53. doi: 10.1016/S0168-6445(03)00047-0. PubMed PMID: 12829274.
14. Chopra I. The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern? J Antimicrob Chemother. 2007;59:587-90. doi: 10.1093/jac/dkm006. PubMed PMID: 17307768.
15. Kim KJ, Sung WS, Suh BK, Moon SK, Choi JS, Kim JG, et al. Antifungal activity and mode of action of silver nano-particles on Candida albicans. Biometals. 2009;22:235-42. doi: 10.1007/s10534-008-9159-2. PubMed PMID: 18769871.
16. Gajbhiye M, Kesharwani J, Ingle A, Gade A, Rai M. Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomedicine. 2009;5:382-6. doi: 10.1016/j.nano.2009.06.005. PubMed PMID: 19616127.
17. Noorbakhsh F, Rezaie S. Shahverdi AR. Antifungal Effects of Silver Nanoparticle alone and with Combination of Antifungal Drug on Dermatophyte Pathogen Trichophyton Rubrum. International Conference on Bioscience, Biochemistry and Bioinformatics. 2011;5:364-7.
18. Montazer M, Alimohammadi F, Shamei A, Rahimi MK. Durable antibacterial and cross-linking cotton with colloidal silver nanoparticles and butane tetracarboxylic acid without yellowing. Colloids Surf B Biointerfaces. 2012;89:196-202. doi: 10.1016/j.colsurfb.2011.09.015. PubMed PMID: 21978552.
19. Dar MA, Ingle A, Rai M. Enhanced antimicrobial activity of silver nanoparticles synthesized by Cryphonectria sp. evaluated singly and in combination with antibiotics. Nanomedicine. 2013;9:105-10. doi: 10.1016/j.nano.2012.04.007. PubMed PMID: 22633901.
20. Rathnayake WGIU, Ismail H, Baharin A, Darsanasiri AGND, Rajapakse S. Synthesis and characterization of nano silver based natural rubber latex foam for imparting antibacterial and anti-fungal properties. Polymer Testing. 2012;31:586-92. doi: http://dx.doi.org/10.1016/j.polymertesting.2012.01.010.
21. Kim KJ, Sung WS, Moon SK, Choi JS, Kim JG, Lee DG. Antifungal effect of silver nanoparticles on dermatophytes. J Microbiol Biotechnol. 2008;18:1482-4. PubMed PMID: 18756112.
22. Hitokoto H, Morozumi S, Wauke T, Sakai S, Kurata H. Inhibitory effects of spices on growth and toxin production of toxigenic fungi. Appl Environ Microbiol. 1980;39:818-22. PubMed PMID: 6769391; PubMed Central PMCID: PMC291425.
23. Jahanshiri Z, Shams-Ghahfarokhi M, Allameh A, Razzaghi-Abyaneh M. Effect of Curcumin on Aspergillus parasiticus Growth and Expression of Major Genes Involved in the Early and Late Stages of Aflatoxin Biosynthesis. Iran J Public Health. 2012;41:72-9. PubMed PMID: 23113196; PubMed Central PMCID: PMC3469004.
24. Pearson SM, Candlish AAG, Aidoo KE, Smith JE. Determination of aflatoxin levels in pistachio and cashew nuts using immunoaffinity column clean-up with HPLC and fluorescence detection. Biotechnology Techniques. 1999;13:97-9. doi: 10.1023/A:1008819914690.
25. Shin SH, Ye MK, Kim HS, Kang HS. The effects of nano-silver on the proliferation and cytokine expression by peripheral blood mononuclear cells. Int Immunopharmacol. 2007;7:1813-8. doi: 10.1016/j.intimp.2007.08.025. PubMed PMID: 17996693.
26. Krishnaraj C, Ramachandran R, Mohan K,

Kalaichelvan PT. Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi. Spectrochim Acta A Mol Biomol Spectrosc. 2012;93:95-9. doi: 10.1016/j.saa.2012.03.002. PubMed PMID: 22465774.
27. Martinez-Gutierrez F, Olive PL, Banuelos A, Orrantia E, Nino N, Sanchez EM, et al. Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. Nanomedicine. 2010;6:681-8. doi: 10.1016/j.nano.2010.02.001. PubMed PMID: 20215045.
28. Yen HJ, Hsu SH, Tsai CL. Cytotoxicity and immunological response of gold and silver nanoparticles of different sizes. Small. 2009;5:1553-61. doi: 10.1002/smll.200900126. PubMed PMID: 19326357.
29. Panacek A, Kolar M, Vecerova R, Prucek R, Soukupova J, Krystof V, et al. Antifungal activity of silver nanoparticles against Candida spp. Biomaterials. 2009;30:6333-40. doi: 10.1016/j.biomaterials.2009.07.065. PubMed PMID: 19698988.
30. Nozari S, Haydari Kohan F, Ashrafi Khozani M, Ahmadi F, Ghasemi Z, Nami S et al. Comparision of antifungal effect of fluconazole alone and in combination with nanosilver particles against candida species isolated from chronic candidal vulvovaginitis. RJMS. 2012;18:8-14. Persian.
31. Marambio-Jones C, Hoek EV. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. Journal of Nanoparticle Research. 2010;12:1531-51. doi: 10.1007/s11051-010-9900-y.
32. Kim SH, Lee HS, Ryu DS, Choi SJ, Lee DS. Antibacterial Activity of Silver-nanoparticles Against Staphylococcus aureus and Escherichia coli. Korean J. Microbiol. Biotechnol. 2011;39:77-85.