

# Inhibitory Effects of Silver Nanoparticles on Growth and Aflatoxin B<sub>1</sub> Production by *Aspergillus Parasiticus*

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## Abstract

**Background:** Aflatoxins (AFs) are secondary hazardous fungal metabolites that are produced by strains of some *Aspergillus* species on food and feedstuffs. Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) 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<sub>1</sub> production of AF-producing *Aspergillus parasiticus*.

**Methods:** *A. parasiticus* was inoculated (10<sup>6</sup> 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<sub>1</sub> 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<sub>1</sub> 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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## 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*).<sup>1</sup> 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.<sup>2,3</sup>

AFs are considered the most carcinogenic, mutagenic, and teratogenic compounds found naturally in foods and feeds.<sup>4</sup> Aflatoxins B<sub>1</sub> (AFB<sub>1</sub>), B<sub>2</sub> (AFB<sub>2</sub>), G<sub>1</sub> (AFG<sub>1</sub>), and G<sub>2</sub> (AFG<sub>2</sub>) are major naturally occurring AFs produced by aflatoxigenic fungi.<sup>5</sup> AFB<sub>1</sub> is the most powerful AF; moreover, AFB<sub>1</sub> has been classified as a human carcinogen in the group 1 by the International Agency for Research on Cancer (IARC).<sup>6,7</sup> In addition, AFB<sub>1</sub> is a hepatocarcinogenic compound that might cause tumors in other organs like colon and kidney.<sup>8</sup> The importance of AFB<sub>1</sub> led to many researches on the effects of different compounds in order to inhibit its production.<sup>9</sup> 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.<sup>10,11</sup> 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.<sup>11</sup> Also silver and its compounds are regarded as a relatively safe antimicrobial metal that has a wide spectrum of activity.<sup>12,13</sup>

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.<sup>14</sup> 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.<sup>15,16</sup> AgNPs have higher effective antimicrobial activity compared to bulk silver metal.<sup>17</sup>

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.<sup>11,18,19</sup>

Antifungal activities of AgNPs against different kinds of fungus have not been reported as much as antimicrobial activities of them by researchers.<sup>16</sup> It was reported that AgNPs could inhibit fungi in low concentrations and those levels had no toxic effect on human cells.<sup>20</sup> 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.<sup>15,21</sup>

The aim of this study was to investigate the effects of AgNPs on the growth and AFB<sub>1</sub> production by *A. parasiticus*.

## Materials and Methods

### *Fungal Strain, Growth Media and Chemicals*

The *A. parasiticus* PTCC 5280, a known producer of AFB<sub>1</sub> was used throughout the study. Potato Dextrose Broth (Merck, Germany) was the culture medium used for AFs production by the fungus.<sup>22</sup> 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<sup>6</sup> spores/well) prepared in sterilized distilled water containing 0.1% Tween80. 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.<sup>23</sup> 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<sup>6</sup> 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.<sup>24</sup> A 50 µl sample loop was used to inject the sample into the HPLC.

## Results

### Antifungal Activities of Silver Nanoparticles on *A. Parasiticus*

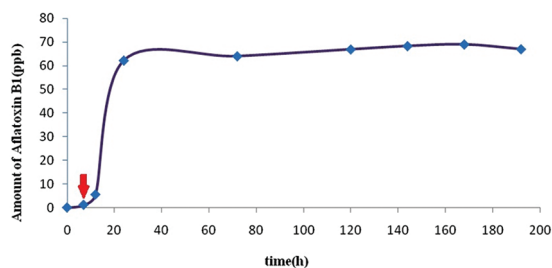
Initial data showed that AgNPs was able to inhibit *A. parasiticus* growth and AF production effectively. Antifungal activity was confirmed for AgNPs (data not shown in details).

### Inhibition of *A. Parasiticus* Growth by AgNPs

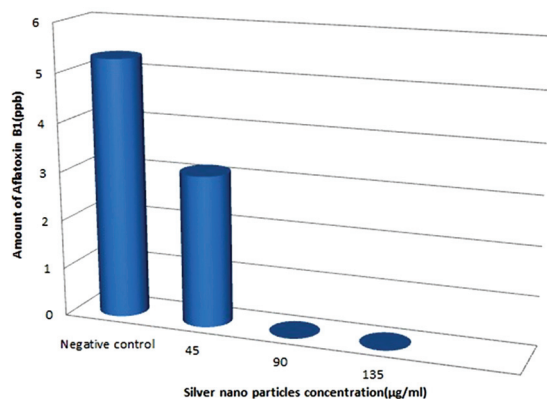
The AgNPs was effectively inhibited fungal growth in microbioassay at some concentrations used. This inhibition was reported in concentration of 180 µg/ml.

### Inhibitory Effects on Aflatoxin Production

Figure 1 illustrates AFB<sub>1</sub> 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<sub>1</sub> production in a dose dependent manner, also AFB<sub>1</sub> inhibition was reported at a concentration of 90 µg/ml after comparing with non-treated controls (Figure 2).



**Figure 1:** Shows Aflatoxin B<sub>1</sub> production by *A. Parasiticus* without silver nanoparticle effect. Arrow: Start time of Aflatoxin production (7 h).



**Figure 2:** Shows different silver nanoparticles concentrations effect on Aflatoxin B<sub>1</sub> 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.<sup>25</sup> Since nano-sized silver materials have stronger antifungal activity than bulk silver materials, it has recently attracted great attention.<sup>20</sup> 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.<sup>26</sup>

Researchers reported the antifungal activity of AgNPs on the same fungus species.<sup>20</sup> Although most of the studies are focused on nanoparticle applications, there are limited studies describing the impact of nanoparticles on human health.<sup>27,28</sup> 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.<sup>20</sup>

Panacek et al. have claimed that nano-sized silver has a significant antifungal activity against *Candida albicans*.<sup>29</sup> Also, in another study by Kim et al., Antifungal effects of AgNPs on *Candida albicans* were investigated.<sup>15,21</sup> 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.<sup>30</sup>

Many studies have shown the antimicrobial effects of AgNPs.<sup>13,31</sup> 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.<sup>32</sup>

Antifungal effects of AgNPs on dermatophytes especially *Trichophyton mentagrophytes*, had been studied by Kim.<sup>21</sup> 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.<sup>16,17</sup> 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<sub>1</sub> are mostly unknown.

Based on previous studies, the main contribution of this study was AgNPs effect on growth and AFB<sub>1</sub> production by *A. parasiticus*.

In the present study, we showed AgNPs inhibiting AFB<sub>1</sub> 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<sub>1</sub> 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<sup>16</sup> and Kim et al. used amphotericin B as a positive control in their study.<sup>21</sup>

To the best of our knowledge, this research was the first study about the effects of AgNPs on AFB<sub>1</sub> production by *A. parasiticus*. Due to the important role of AF contamination, especially negative effect of AFB<sub>1</sub> 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<sub>1</sub> 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.

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