

## Original research

### MgO-SiO<sub>2</sub> Nanocomposite: For Adsorption of Aflatoxin in Wheat Flour Samples

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### ABSTRACT

Aflatoxin is a fatal toxin that causes liver tumor and hepatitis, and is produced mostly by *Aspergillus flavus* on food and culture media. In this in vitro lab trial study we used nanocomposite Magnesium Oxide-Silicon Dioxide (MgO-SiO<sub>2</sub>) for aflatoxin adsorption in wheat flour samples. *A.flavus* was isolated from decaying bread, and incubated for one week in room temperature. The produced aflatoxin was extracted by chloroform, and thin-layer chromatography was carried out for aflatoxin detection. Ten titers (5-1000 ppm) of extracted aflatoxin were prepared and added to 1 gram of wheat flour in separate tubes and incubated at room temperature for 30 min. Then 1 mL of nanocomposite MgO-SiO<sub>2</sub> was added to each tube, shook for 30 min, and washed 3 times with saline. The amount of aflatoxin in each sample was measured by high performance liquid chromatography method. It showed that nanocomposite MgO-SiO<sub>2</sub> was an effective adsorbing agent for aflatoxin, and the amount of reduction is related to aflatoxin concentration, i.e., 100% removal for 5-20 ppm, about 95% removal for 40-100 ppm, and about 80% removal for 200-1000 ppm. If the results could be repeated by other investigators, it is hoped that MgO-SiO<sub>2</sub> may become industrially and medically of widespread use for removal of aflatoxin in stockpiled wheat and flour. Other mycotoxins may be treated by similar methods.

### Keywords

Nanocomposite, Aflatoxin, Adsorption, MgO- SiO<sub>2</sub>

### Introduction

Many fungi in the nature may easily grow on the food stuff which is used by humans or animals [1]. Some of these fungi can produce toxins, hence causing mycotoxicosis, the consequences of which range from headache and nausea to hepatitis (inflammation of liver), hepatic cirrhosis (liver failure), cancer, and even death [2]. Estimates made by Food and Agriculture Organization (FAO) show contamination of 25% of cereals with mycotoxins each year. This huge amount shows its importance and need for global solutions [3].

Aflatoxin is a lethal poison mainly produced by *A.flavus* and *A. parasiticus* on food or culture media. It may induce liver failure and malignant tumor (hepatocellular carcinoma) [4]. Four main types of this toxin are B1, B2, G1, and G2. Aflatoxin B1 has the most toxicity and carcinogenicity [5], and is rapidly absorbed in the gastrointestinal tract [6]. Through the blood circulation, it is distributed in the body, including milk, in which it is known as aflatoxin M1, and can harm the babies of human and other animal species [7].

The growth of *A.flavus* and aflatoxin production depends on temperature and humidity. When the storage conditions in cereal pools (as in silo) are not standard, this toxin will be accumulated on them [8]. The economic effects of this contamination on the agriculture sector is enormous, because it results in reduced nutritional value of foodstuff, decreased meat production from animals, and toxicity in users of dairy products. Since the people use large amounts of milk or its derivatives each day, the adverse consequences of aflatoxin are of utmost importance from a medical point of view [9, 10].

The preventive measures include providing suitable piling conditions for cereals, use of chemicals (like ammonia) for fight against fungi, and boiling the milk. However, despite all of them, there is still some aflatoxin in dairy products [11]. Aflatoxin-adsorbing agents, which bind it and prevent

from its uptake by gastrointestinal cells, are apparently useful for decreasing blood exposure to this toxin. Adsorbent agents for aflatoxin include aluminosilicates, activated charcoal, cell walls of fungi and bacteria, and polymers [12]. Also, some microorganisms are capable of changing aflatoxin to some harmless compounds by special enzymes, collectively known as mycotoxin biotransformers [13, 14].

There have been few studies using nanoparticles for adsorption of aflatoxin. The purpose of this in vitro laboratory trial research was to evaluate the capacity of nanocomposite MgO-SiO<sub>2</sub> for adsorbing aflatoxin in contaminated wheat flour samples. The potential applications of such composites, if found in future studies to be hygienic and devoid of bioenvironmental hazards, may include its routine use in storage pools of wheat and flour.

### **Materials and methods**

#### **Preparation of aflatoxin**

We isolated the fungus *A.flavus* from decaying bread, and inoculated it on potato dextrose agar medium in room temperature for one week. After microscopic confirmation of the proper fungal genus and species, then chloroform was used for extraction of the produced aflatoxin. Determination of the presence of aflatoxin was done by thin-layer chromatography (TLC), in which 10 microliters of the extract was placed on silica gel plates, and after drying it was transferred to the TLC tank (containing 50 mL of chloroform/methanol with the ratio of 98/2). After passage of the solution on the plate, it was allowed to dry, and was studied under ultraviolet light at 365 nm. A blue fluorescence was proof for presence of aflatoxin.

#### **Titration of aflatoxin and mixing with wheat flour:**

Ten titers (5-1000 ppm) of the aflatoxin extract were prepared in test tubes by 95% ethanol, and 1 mL from each dilution was added to 1 gram of wheat flour obtained from the main silo in Yazd, Iran.

### **Preparation of the nanocomposite:**

Nanoparticles of Magnesium Oxide (MgO) and also Silicon Dioxide (SiO<sub>2</sub>) were purchased from NST company, China, and were mixed to the ratio of 40/60. A homogenous solution of 100 grams of this composite was added to distilled water, to prepare a final volume of 1000 mL (0.1 g/mL concentration). Atomic force microscopy (AFM) was used to confirm the molecular structure of the nanoparticles.

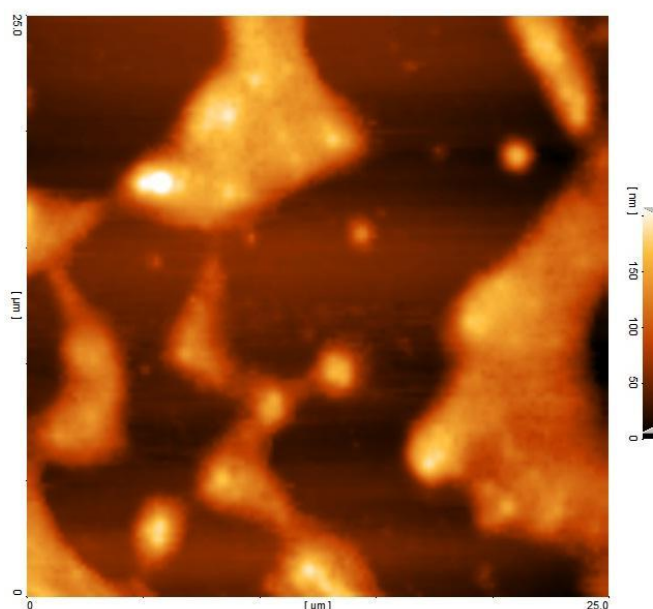
### **Aflatoxin adsorption test:**

After 30 minutes incubation of aflatoxin-contaminated wheat flour in room temperature which allowed it to dry (i.e., all of the aflatoxin was absorbed by the flour), 1 mL of the nanocomposite MgO-SiO<sub>2</sub> was added to each tube and shook for 30 minutes. To remove extra (unused) nanocomposite, a saline (9 g/L NaCl) solution was added to each tube for 3 times, the tube was centrifuged and the supernatant was discarded. Then 1 mL of distilled water was added to the contents of each tube, and they were studied by high performance liquid chromatography (HPLC) method to determine the amount of unadsorbed aflatoxin. To validate the whole process, the concentration of aflatoxin with and without nanocomposite was determined and compared, that is, we used the same series of 10 tubes containing wheat flour and aflatoxin, but without nanocomposite, to act as positive control. Also, another tube containing wheat flour and nanocomposite, but without aflatoxin, served as a negative control. All of the tests were duplicated to increase the accuracy of results.

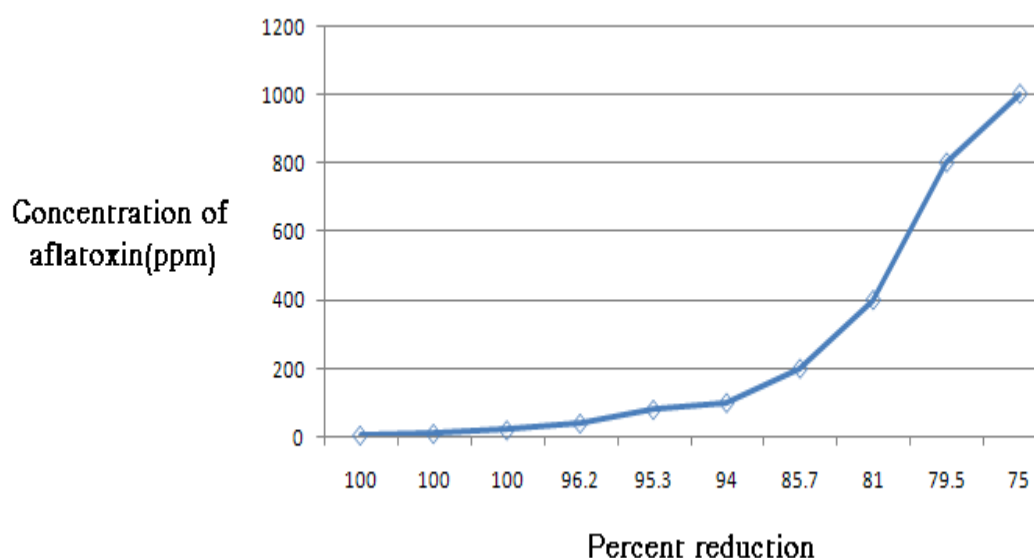
For HPLC method, 1 mL of the HPLC-grade 40% methanol was added to each of the above-mentioned test tubes, vortexed, and the contents were passed through 0.45 micrometer filters to yield a clear solution. A 100 microliter extract from each tube was injected to the HPLC system (model Waters 695, USA) which was equipped with a fluorescent detector (excitation wavelength=360 nm, emission wavelength=420 nm). The speed of the moving phase was 1 mL/min, at 100  $\mu$ A flow intensity. All samples passed the columns in the HPLC system, and the curves for each specimen were plotted. The area under each (according to the standard curve) curve was calculated to determine the amount of aflatoxin.

### Results and Discussion

In the images taken by AFM (model DMF, Denmark), the size of nanoparticles was between 50 and 150 nm. [Figure 1]. The results obtained by HPLC before and after addition of nanocomposite were as follows [Table 1]. Figure 2 demonstrates the reduction curve on a linear-linear paper.



**Figure 1** AFM photomicrograph of nanocomposite MgO-SiO<sub>2</sub>.



**Figure 2** The graph of reduction of aflatoxin in wheat flour samples.

**Table 1** The amount of reduction of aflatoxin in wheat flour samples.

Concentration of aflatoxin(ppm)	5	10	20	40	80	100	200	400	800	1000
without addition of aflatoxin										
Concentration of aflatoxin(ppm)	0	0	0	1.5	3.7	4	28.6	76	163.5	250
with addition of nanocomposite										
Percent reduction	100	100	100	96.2	95.3	94	85.7	81	79.5	75

The equation for calculation of the percent reduction of aflatoxin is:

**Equation 1.**

$$\%reduction = [without\ nanocomposite] - [with\ nanocomposite] / [without\ nanocomposite] \times 100$$

The results show that the MgO-SiO<sub>2</sub> nanoparticles are efficiently capable of adsorbing aflatoxin, and reducing it in wheat flour. However, the adsorbing capacity depends on (is roughly inversely proportional to) the amount of aflatoxin, so that in concentrations of 5-20 ppm it is 100%, but it falls to 75% in concentrations of about 1000 ppm. One of the main applications of nanoparticles has been their use for adsorbing various compounds, because of their high surface area compared to larger molecules. The adsorption characteristics of the nanocomposite MgO-SiO<sub>2</sub> depends on the concentration of aflatoxin in the specimens, and may reach a saturation level. Since the surface-to-volume ratio in nanoparticles determines the adsorption capacity, it seems possible to achieve higher capacities by decreasing their size. Also, it may be possible to increase that capacity by using higher concentrations of nanoparticles (we used 0.1 g/mL).

There has been no previous report on usage of MgO-SiO<sub>2</sub> nanoparticles for aflatoxin, but montmorillonite nanocomposite (MMN) was applied. Findings suggested that MMN particles can effectively reduce the toxicity of aflatoxin and be a potential ameliorator of aflatoxicosis in broiler chicks (15). The current study depicted that liquid-phase nanoparticles also have high potency for adsorption of aflatoxin. If these results could be repeated by other investigators, it may be that MgO-SiO<sub>2</sub> becomes industrially and medically of routine use for removal of aflatoxin in stockpiled wheat and flour. Obviously, other mycotoxins may be treated in the same way, and other cereals could be managed in a similar manner, hence increasing food safety in the world.

The most important aspect of usage of nanocomposite on foodstuff is their safety and biocompatibility, since they become attached to food, and can be released or adsorbed in the intestines. So, future research is needed to assess the effects of these nanoparticles on the alimentary tract, blood cells, and other cells.



### Conclusion

In this in vitro lab trial study we used nanocomposite MgO-SiO<sub>2</sub> for aflatoxin adsorption in wheat flour. It showed that nanocomposite MgO-SiO<sub>2</sub> was an effective adsorbing agent for aflatoxin, and the amount of reduction is related to aflatoxin concentration, i.e., 100% removal for 5-20 ppm, about 95% removal for 40-100 ppm, and about 80% removal for 200-1000 ppm.

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