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RESEARCH ARTICLE

# Evaluation the Efficiency of Magnesium Oxide Nanoparticles in the Reduction of Ochratoxin A and in the Inhibition of *Asperillusniger*

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## Abstract

This study was conducted in Mycotoxins Laboratory /Department of Plant Protection/College of Agriculture at" University of Baghdad". This study aimed to determine the efficiency of magnesium oxide nanoparticles (MgO) (NP) in the inhibition of *Aspergillusniger* growth and in the degradation ocra a toxin. The results found that MgOnanoparticles significantly inhibited the growth of *A. niger*. There were significant differences between all the concentrations of MgOnanoparticles in comparison to control groups, while no significant difference was fund between the concentration of 2% and 3%;a significant differences also was detected between the concentrations (2 and 3)% for the first concentration (1%) and the 95% for concentration of(3%). Moreover, the results observed that MgO nanoparticles was potential in the reduction of ocra Atoxin A that produced from *A. niger*using Performance liquid chromatography (HPLC) technique; the percentage of reduction of toxin were 90.1%, 92.2%, 93.7% for concentrations of NaO2 1%, 2% and 3% respectively, however the best reduction percentage was 92.2% with concentration of 0.06 µg/kg after the treatment with MgOnanoparticles at 2%.

**Keywords:** *Ochratoxin A, MgO (NP), inhibition of Aspergillusniger, Reduction.*

## Introduction

*Aspergillusniger* is one of the most common fungi found in different regions of the world, especially in the warm regions [1]. It also contains about 250 species of fungi [2]. This genus of fungi causes many types of diseases in plants, humans and other species. Furthermore, their speciesproduce toxin as well as it is used in important industries such as production of citric acid and enzymes. *A.niger* is an industrial fungus used to produce citric acid, oxalic and geoconic since 1919 as well as its production of many types of enzymes including pectinase, amylase, dlycoamylase, invertase and lactase; also it was used in industrial fermentation [3].

*A. niger* was classified as a safe living organism Generally Nizes As SafeReceg (GRAS) by US Food and Drug Administration [4]. The safe use of *A. niger* has been resulted from the long history of its use in the food industry to produce many enzymes and organic substances [5]. Interestingly, numerous studies have been indicated the susceptibility of *A. niger* in the production of ocraA toxin [6, 7]. Several studies have been based on the health effects of ocraAtoxin in living organisms. It is considered to be a second class of carcinogenic toxins (Group 2B) and its main effect in mammals especially in the kidney (Nephrotoxic) as it acts on the deterioration of the renal tubules function leading to the emergence of symptoms of diabetes and syphilis in humans; it's also effect on the liver (Hepatotoxic) and causes congenital malformations of the embryos (Teratagenic toxic).

The toxin is a weak mutagenic toxin as well as its effect in the suppression and inhibition of immune system [8, 9].Recently, nanotechnology was used in agriculture and food field [10, 11]. Nanotechnology is a modern technology due to its high efficiency and limited economic cost due to low

concentration that used in various disciplines. Therefore, in the last century it is become the most important and most exciting science, with its great hopes and scientific resources that will change the face of the world in the near future [12]. Recent studies have been confirmed that nanoparticles have special physical and chemical properties due to their small size (< 100nm), their variety of shapes and the way of surfaces arrangement, enabling them to interact in a manner compatible with biological systems [13].

Studies have been also observed the effective action of magnesium oxide nanoparticles in inhibiting the growth of some pathogenic fungi and reducing the toxins production including aflatoxin B1 produced by *Aspergillusflavus* and ocratoxin by *A. niger* [14, 15, 16, 17]. The aim of this research is to use environmentally friendly materials such MgO nanoparticles in the inhibition of *A.niger* growth and reducing of ochraA toxin production from it.

## Materials and Methods

### Fungi Isolation

*A. niger* isolate was collected directly as pure diagnosed isolate genetically using PCR technique from Mycotoxins Laboratory (Prof. Halima ZaghirHussain/Department of Plant Protection/Faculty of Agriculture /University of Baghdad). This isolates cultures on potato extract and the dextrose (PDA) medium; this medium was prepared by dissolving 39 gm of medium in 1 liter of distilled water, then sterilized using autoclave at 121° C and pressure 1.5 kg/cm$^2$ for 20 min, and after that tetracycline was added to the medium at concentration of (250 mg/L) to prevent the growth of bacteria; the medium was placed into Petri dishes (9 cm) and inoculated with *A. niger* isolate and kept until using for subsequent experiments.

### Preservation of Fungi Isolates

*A. niger* isolates cultured on PDA medium for 7 days and all the isolates were kept in sterile test tubes contain 10 ml of PDA medium and placed in an oblique form. Then, all tested tubes were inoculated with *A.niger* using cork puncher and incubated at a temperature of 25 ± 2C$^0$ for 5 days and being stored in the refrigerator until using.

### Determination of the Susceptibility of *A. niger* that Cultured on Yeast-sucrose Liquid Extract Medium in the Production of Ochratoxin A *in Vitro*

Yeast and sucrose extract medium was prepared and the liquid sucrose by dissolving 39 g/L in distilled water, then medium was distributed in glass flasks with a capacity of 300 ml (100 ml of medium in each flask);all flasks with medium were sterilized in autoclave at 121$^0$ C and pressure of( 1.5 kg/cm$^2$) for 15 minutes. All the flasks were inoculated with 4 discs of *A. niger* which cultured on PDA medium for (5-7) days, then the inoculated flasks incubated at 25° C± 2° C for 21 days [18].

### Extraction of Ochra toxin A from Yeast-sucrose Extract Liquid Medium

Ochra toxin A was extracted from the liquid medium according to [19] method as following:

- Suspension was filtered using WahtmanNo.4 filter paper and 20 mol transferred to a 250 ml separating funnel.
- 40 ml of chloroform was added to the separation funnel and the mixture mixed and the lid opened periodically to remove the accumulated gases in the separation funnel (at least twice), then the funnel left on the holder for 15 minutes until two layers separate.
- The lower layer (chloroform layer) was collected in vials and added to the funnel, and (10) ml of chloroform added again and mixed well with expulsions of gases; the funnel left on the holder until two layers separated.
- The chloroform layer (bottom layer) was collected in vials and dried at a 40° C, and then coated with aluminum foil from the outside after closing well to prevent the exposure to light and kept in frozen (-20° C) until using.

### Preparation of the Standard Solution of ochra toxin A

The standard solution of ochra toxin A was obtained from Dr. Iyad Abdul Wahid Al-Hiti(Sigma chemicals company) at a concentration of (2 µg/L), it was prepared by dissolving it with 1 ml of chloroform with stirring, and preservedit at -18 ° C until use.

### Extraction and Estimation of Ochra toxin A Concentration using High

**Performance Liquid Chromotography (HPLC)** Technique

Ochra toxin A was extracted according to [20] method as fallowing:

- 2 g of the living mass of *A. niger* was taken from liquid yeast medium and left to dry in the oven at 40° C for 24 hours, and then grinded and placed in 250 ml glass flask.
- Mixture of 25 ml of methanol with 25 ml of chloroform was added to the dried *A. niger*.
- The whole mixture placed on the shaker for 2 hours for the purpose of homogenization.
- The samples were filtered by using Whatman No.2 filter paper, and the e was transferred to the separation funnel and 25 ml of methanol (90%) added to the suspension and left for separation.
- The filtered mixture transferred to the separation funnel and (25) mlof hexane with 25 ml of methanol (90%) was added, and left in the funnel for separation.
- The bottom layer which containing methanol and left to dry in a water bath.
- The dried sample was collected and then 25 ml of chloroform with 25 ml of distilled water added in the separation funnel, the washed twice with distilled water after shaking the funnel and leave it until the two layers are separated, while the top layer is discarded.
- The lower layer (chloroform) was passed on the filter paper that containing 10 g of anhydrous sodium sulphate.
- The filtered mixture was taken and leave to dry.

10. 3 ml of acetonitrile was added as a solvent and all the bottles were covered with aluminum foil from the outside to prevent the exposure to light and keep it as frozen until detection of toxin by HPLC technique.

**Detection of OchratoxinAin Yeast Extract Using HPCL Technique**

- Stander solution of toxin was prepared by a concentration of (2) ppm.
- The samples were analyzed in laboratories of the Ministry of Science and Technology / Department of Environment and Water using a High Performance Liquid Chromatograph (HPLC) technique using (HPLC) system (Sykam, Germany). The device system consists of the mobile phase which is a mixture of water and acetonitrol (40:60) ml, and the stationary phase that consisting of a column made up of stainless steel consisting of silica particles, and the column that uses was C18 (25cm * 4.6m * 5um) type with flow rate (1ml / min) and using Fluorescence detectors:

EX = 365 nm / Em = 435 nm and temperature (30° C).

3. A 5 µl of standard toxin injected followed by the injection of samples according to the retention time of each sample and the concentration of toxin in each sample calculated according to the following equation:

$$\frac{\text{coentration of standard toxin x area of sample curve}}{\text{area of standard toxin curve}} \times \frac{\text{Dilution factorc}}{\text{weight of sample}}$$

Then, the reduction percentage was calculated according to the following equation (9):

$$\frac{\textit{Toxin cocentration in control} - \textit{toxin concentration in sample}}{\textit{Toxin cocentration in control}} \text{X } 100$$

**Preparation of Nanoparticles Solution**

MgO nanoparticles supplied from MTI (U.S.A) and were with following properties as shown in Table (1):

**Table 1: characteristics of MgO nanoparticles**

| ***Material*** | ***Magnesium OxidNanoparticies*** |
|---|---|
| Purity | 99.9% |
| APS | 40 nm |
| SSA | ~ $40^{m2}$/g |
| Morphology | Polyhedral |

| | |
|---|---|
| Bulk density | 1.2 – 1.3 g/cm3 |
| True density | 3-58 g/cm3 |

The size of MgO nanoparticles was examined by using Zeta potential device in the laboratories of the Environment and Water Department/Ministry of Science and Technology. The weights (1.21 / 2.24 / 3.63) g was used for MgO nanoparticles; 100 ml of distilled water was added for each of these weights to prepare several concentrations (1%, 2% and 3%) respectively.

Sample homogenized using Ultrasonic Homogenizer (20-20,000Hz) for 30 minutes; this device produces ultrasound wavwsat a frequency (20-20,000Hz) to ensure good mixing (21) to maintain the size of nanoparticle and ensure uniform distribution in the mixture. All concentrations of toxins placed individually to glass flasks (100 ml)contains (2.4) g of PDA medium PAD and (30) ml of distilled water; then this mixture sterilized in autoclave at 121° C and pressure 1.5 kg/cm $^2$ for 20 minutes and then the mixture pour off before hardening in petri dishes with (9)cm in diameter.

### Estimation the Efficiency of MgO Nanoparticles in Inhibiting the Growth of *A. niger* on PDA Medium

All dishes were inoculated with *A. niger* isolate that collected from PDA medium after( 7 days) of growth, and incubated in the incubator at"25± 2° C" for one week, and using control group involved dishes without *A. niger* for comparison; the percentage of inhibition zone was calculated according to the following equation:

$$\frac{\text{mean of inhibition zone of control} - \text{mean of inhibition zone of sample}}{\text{mean of inhibition zone of sample}} \times 100$$

### Estimation the efficiency of MgO nanoparticles in the degradation of ochra toxin Aproduced by *A. niger*.

Yeast-sucrose extract liquid medium was prepared and 100 ml of medium placed in glass flasks with a capacity of 200 ml, and sterilize in autoclave. All the flasks inoculated with 0.5 cm in diameter from the margin of *A. niger* growth; MgO nanoparticles was added in three concentrations (1%, 2%, 3%) with 3 replicates for each concentration. The flasks were then incubated at 25 ± 2° C for 21 days (18).Ochra toxin A was extracted from the filtered suspended of medium.

## Results and Discussion

### Quantitative Determination of "Ochratoxin A" using High Performance Liquid Chromatography (HPLC) Technique

The results of quantitative estimation of toxin using HPLC showed that *A. niger* isolate was able to produce ochratoxin A at a concentration of 1.18 µg/g, and this is in agreement with the studies that confirmed the ability of *A. niger* to produce Ochratoxin A with high concentrations [23].

### Estimation the Efficiency of MgO nanoparticles in the Inhibition of *Aspergillusniger* Growth in Vitro

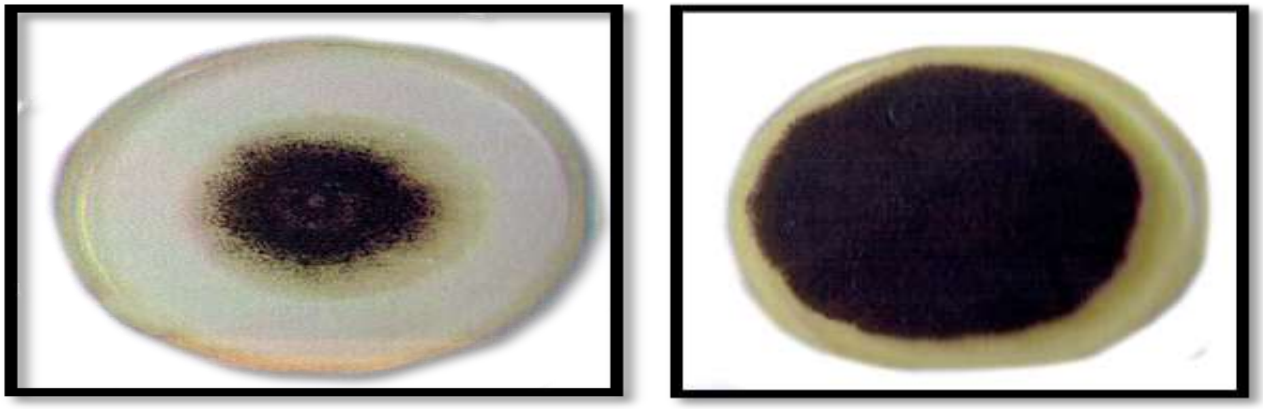
The results have been shown the effectiveness of MgO nanoparticles in inhibiting the growth of *A. niger* in vitro at concentrations (1%, 2% and 3%)(Table 2). The inhibition percentage onPotata dextrose agar (PDA) medium was 88.3%, 95.1% and 94% respectively (Fig. 1). These results are consistent with other studies that indicated the high effectiveness of nanoparticles and their oxides in inhibiting many pathogens of organisms [12, 24].

**Table 2: Estimation the efficiency of MgO nanoparticle in the inhibition of *Aspergillusniger* growth on PDA medium**

| Concentration % | Mean | Inhibition percentage (%) |
|---|---|---|
| 1 | 0.11 | 88.3 |
| 2 | 0.12 | 95.1 |
| 3 | 0.12 | 94 |
| L.S.D value | 0.02 | 2.04 |

Interestingly, [14] confirmed the high efficiency of MgO nanoparticles, and the inhibition percentage was 100% with a concentration of 2% and 3%, while with concentration of 1% was 95.53% in the inhibition of *A.flavus* in stored maize seeds.

The reason for inhibition properties of MgO nanoparticles against the pathogenic fungi is due to the surface area of the nanoparticle surface and the sharp structure of the nanoparticle surface, whereas the surface area of the nanoparticle works to absorb the fluid in the nutrient medium and thus makes it unsuitable for the growth of the fungi and inhibits their growth. Moreover, the sharp structures of the nanoparticles surface area make it able to lyse the wall of the host cells and thus exiting the cell components lead to inhibit the growth of fungi [12, 24].



**Concentration (2%)** **Control**

**Figure 1: The effect of MgO nanoparticles in the inhibition of *A. niger* growth on PDA medium**

### Effect of MgOnanoparticles in the Reduction of ochra Toxin a Production from *Aspergillus niger*

The efficiency of MgO nanoparticles (NP) in the reduction of ochratoxin A produced by *A.niger*, was evaluated using High Performance Liquid Chromatography (HPLC) technique; the result revealed that MgO nanoparticles was able to reduce the production of ochra toxin A and the reduction percentage was (90.1%) with concentration of (0.06)µg/kg with (2%) of MgO nanoparticles, while the reduction percentage was (92.2%) with concentration at (0.06) µg/kg with (2%) of MgO. The inhibition percentage was (93.7%) and with concentration of (0.07) µg/kg after the treatment with MgO nanoparticles at (3%) of concentration in comparison to control group which was (0.13)µg/kg; these results statistically were with significant differences between treatments (Table 3). These results are in agreement with [25] who demonstrated the efficiency of nanoparticles such as $SiO_2$, MgO and ZnO in inhibiting and reducing fungal toxins. As concurred with the findings of [26] and [27] which found that nanoparticles potentially act as agents in adsorption and destruction of fungal toxins.

**Table 3: the effect of MgO nanoparticles in the degradationof ochra toxin a produced by *Aspergillusniger***

| Type of treatments | Concentration of ochra toxin A (µg/kg) | The reduction percentage (%) |
|---|---|---|
| MgO nanoparticles (1%) | 0.06 | 90.1 |
| MgO nanoparticles (2%) | 0.06 | 92.2 |
| MgO nanoparticles (3%) | 0.07 | 93.7 |
| Control group (*A. niger*) | 0.13 | 0.00 |
| L.S.D value | 0.01 | 2.01 |

*each number referred to the mean of three replicates

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