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# PREPARING NANOPARTICLES USING SOME BIOLOGICAL METHODS AND STUDYING THEIR EFFECT ON SOME MYCOTOXINS

**Zahraa Essa Radhi**[1]
Environmental Research and Pollution Prevention Unit, College of Science, University of Al-Qadisiyah, Iraq.
zahraa.Essa@qu.edu.iq

**Zahraa Falah Azeez**[2]
College of Biotechnology, University of Al-Qadisiyah, Iraq.
zahraa.falah@qu.edu.iq

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**Abstract: General Background:** Mycotoxins, toxic substances produced by certain fungi and bacteria, pose significant health risks to humans and animals, necessitating effective remediation strategies. **Specific Background:** The use of environmentally friendly approaches to mitigate the impact of these toxins is crucial. Nanotechnology, particularly in conjunction with plant extracts, has emerged as a promising method for toxin remediation due to its cost-effectiveness and efficiency. **Knowledge Gap:** Despite the potential of nanomaterials in mycotoxin management, there is limited research exploring the interaction between plant extracts and nanotechnology in inhibiting toxin-producing fungi. **Aims:** This study aims to investigate the efficacy of zinc oxide nanomaterials synthesized from mint plant extracts in mitigating the effects of mycotoxins and inhibiting the growth of mycotoxin-producing fungi. **Results:** Mint leaves were collected from various locations in Al-Qadisiyah Governorate over an eight-week period. The extracted plant materials were used to synthesize zinc oxide nanoparticles, which demonstrated significant inhibitory effects on the growth of specific fungi responsible for mycotoxin production, thereby reducing mycotoxin formation. **Novelty:** This research highlights the innovative application of mint-derived zinc oxide nanoparticles as a dual-action agent—suppressing fungal growth while simultaneously preventing mycotoxin synthesis. **Implications:** The findings provide valuable insights into sustainable agricultural practices and mycotoxin management, paving the way for the development of natural, eco-friendly solutions to enhance food safety and protect public health.

**Keywords**: Mycotoxins, Nanotechnology, Zinc Oxide, Plant Extract, Fungal Inhibition, Al-Qadisiyah.



## Introduction

Mycotoxins are toxic by-products produced by some species of filamentous fungi and have long-term negative effects for humans and animals alike. Although therapeutic methods are constantly improving, the best method is nanotechnology, which has been used in all fields due to its low cost. Its effectiveness in eliminating fungal toxins (Horky et al.,2018).Some types of fungi secrete some secondary metabolic substances that may have a harmful effect on animals and humans,These secondary substances are called mycotoxins (Turner et al.,2010).In view of most of the old methods of inhibiting the growth of fungi, researchers have been proposing new ideas and methods to inhibit the growth of fungi and mycotoxins by using nanoparticles (Jogee & Rai.,2020).Mycotoxins have serious effects on health (such as cancer, birth defects, and genetic mutations), and this represents a global concern,To ensure human health, mycotoxins must be monitored, because in recent years there have been many poisoning incidents due to mycotoxins (Yang et al.,2020).The most important types of fungi that produce mycotoxins are Aspergillus species and Fusarium species Includes Aspergillus {ochratoxin A (OTA), aflatoxins (AFL), trichothecenes and deoxynivalenols (DON)) , The most important mycotoxins are aflatoxins, and the most important types of these toxins are B1, B2, G1, and G2.It is produced by two types of fungi, Aspergillus parasiticus and Aspergillus flavus, which are abundant in grains, soil, and decaying plants( Elkenany & Awad.,2021).They are classified according to the value of the flow rate (rF) on thin layer chromatography (tlc) slides, as well as according to the color of their fluorescence under ultraviolet rays (Qi et al.,2015).but Fusirium includes {fumonisins (FUM) B1 and B2,zearalenone (ZEA)} and the emerging mycotoxins fusaproliferin., moniliformin, peofercin, Eniatin), Clavisceps (ergot alkaloids), and Alternaria (alternariol, alternariol, alternariol methyl ether, altertoxin, and tenosonic acid) (Cunha et al.,2018). For many years, the mechanism of action of mycotoxins and their dangerous effects have been studied in terms of disorders of the central nervous system, poisoning of the heart, liver, and digestive system, as well as kidney poisoning (Sobral et al.,2018; Dellafiora et al.,2018; Freire and Sant'Ana.,2018).Mycotoxins have a toxic effect on animal species.Poultry is considered the most sensitive, then pigs and ruminants (Cheat et al.,2016).With almost 100% efficiency, ruminants metabolize some types of mycotoxins (Rodrigues.,2014). Because mycotoxins are lipophilic, they are preferred to be part of animal products (Krstanovic et al., 2017). For example, AFL M1 toxin easily enters milk, which may cause significant health effects (Aslam et al., 2015). As a result of food contaminated with these mycotoxins, it may lead to acute or chronic poisoning of animals and humans (El-Sayed et al., 2022).

Nanotechnology offers many solutions to control many pathogens as it has environmentally friendly advantages (Gacem et al.,2020).Many nanomaterials have been used to treat mycotoxins through the rapid development of this type of technology (Jiang et al., 2018).In the past, there has been an increase in nanotechnology due to its use in several applications such as medicine, chemistry, etc (Pelaz et al.,2012).Most recent studies show that the green synthesis of metals is of high importance, as metal oxides such as zinc, copper, silver, gold, and nickel are gaining ground (Moodley et al.,2018;Suresh et al.,2018). Among various metal oxides, ZnO nanoparticles exhibit high electronic mobility, large exciton binding energy, wide band gap, and high optical transmittance (Kołodziejczak-Radzimska & Jesionowski .,2014).Metal oxides and nanostructures exhibit chemical and biophysical properties that include extraordinary optical properties, catalytic activity, thermomechanical properties, and antimicrobial activity against pathogenic microbes (Geethalakshmi & Sarada.,2013; Aiad et al.,2014).Nanoparticles acquire multiple properties due to their small size, which makes them desirable for many uses, such as cosmetics (Raj et al., 2012).Recently, it has been observed that there is great interest around the world in the antifungal activity of zinc oxide nanoparticles (ZnO NPs), due to the emergence of severe drug resistance by microorganisms Nanomaterials have clearly shown high activity against multidrug-resistant microorganisms (Ashour et al., 2020; El-Tarabily et al., 2021). Most types of food, after being exposed to contamination or unsuitable conditions, contain fungi capable of producing a toxic substance or a group of toxins that are harmful to human and animal health ElHamaky et al., 2016).

The mechanism of action of the chemicals present in the plant extract is as a reducing agent to convert mineral precursors into nanoparticles. These materials are characterized by being non-toxic and acting as antioxidants (Singh et al.,2018) The most important phytochemicals that contribute to the reduction process are aldehydes, alkaloids, and phenolic compounds. Also, the concentration of mineral salts, pH, and temperature affect (stability, quantity, and synthesis of nanoparticles) (Mukunthan & Balaji.,2012).

The aim of this study is to control fungi that secrete mycotoxins using environmentally friendly nanoparticles.

## Methods

### 2.1- Collect plant samples

Mint plants were collected from some grocery stores and gardens located in Al-Qadisiyah Governorate and from various places (Al-Diwaniyah, Afak District, Al-Shamiya District, Al-Shinafiya District, Al-Daghara District, and Al-Nouriya District) on 2/3/2024 to 3/29/2024.

### 2.2-Preparation of plant extract from mint leaves

The plant extract is first prepared by collecting the leaves of the plant used for study and removing all dust and particles stuck in it by washing with distilled water, then drying it at room temperature,Then we weigh 100 grams of it, cut it into small parts, add a little water to it and grind it using a mortar and pestle to soften the cell membrane.The process is done the extract is boiled for 15 minutes and in order to remove the broken cell parts, it is filtered with filter paper, then we use a centrifuge at 2400 rpm for 5 minutes, then we use the pure extract to synthesize the required nanoparticles (Jayachandran et al.,2021).

### 2.3-Synthesis of zinc oxide nanoparticles

Zinc nitrate was added to the previously prepared plant extract and for 20 minutes, the mixture was stirred at a temperature of 65 degrees Celsius. Then we began to notice the color changing from green to light yellow. After that, it was left at the same temperature overnight until we obtained a thick yellow paste. It was left to dry and calcined at a temperature of 400 degrees. At °C, the impurities in the sample are removed by calcination, after which we obtain pure zinc oxide nanoparticles (Nayak et al.,2020).

### 2.4-The mechanism of action of nanoparticles to inhibit toxins or toxin-producing fungi

The most important thing that distinguishes nanoparticles is their extremely small size, as they work to penetrate cell membranes, which leads to their damage, that is, they deliberately form holes on the surface of the cells, thus accumulating them and leading to cell death due to the disturbances occurring in the fungal hyphae as a result of the effect of nanoparticles on the sugar and protein content in the fungal hyphae (Jogee.,2020; Prabhu & Poulose.,2012; Ouda.,2014).

### 2.5-Isolation and identification of mycotoxin-producing fungi

A portion of the samples is taken and placed on PDA medium containing 0.05 mg of Tetracycline and incubated at a temperature of 25 degrees Celsius for a period ranging from one to five days, after which we observe the growth of different types of fungi that are diagnosed according to their morphological appearance in terms of the shape of the colonies, conidia, and pods according to ( Atef et al.,2019; Pitt & Hocking.,2009).

### 2.6- Using zinc oxide nanoparticles at different concentrations on some types of toxin-producing fungi.

Several concentrations (550 µg/mL. 600 µg/mL. 650 µg/mL. 700 µg/mL. 750 µg/mL. 800 µg/mL) of previously prepared zinc oxide nanoparticles were used, with distilled water added to them to make a diluent. After that, three replicates were made for each concentration, and 1 ml of each concentration was taken and added before pouring the PDA medium into the pouring dishes. After pouring the medium and solidifying, the plates were inoculated with the isolated fungi, and Tetracycline antibiotic was used on some of the plates as a positive control. Then the plates were left untreated and containing the fungal samples as a negative control, The incubation process was carried out at a temperature of 25 degrees Celsius for a period of (1-5) days.

## Results and Discussion

### 3.1-Isolation and diagnosis of some types of fungi that produce mycotoxins

The table below shows the number of samples taken for the purpose of the study that were grown on PDA medium containing 0.05 mg tetracycline/ml for a week and at an appropriate temperature. 15 samples were taken from corn fruits, so the number of infected samples was only two, i.e. 13.3 of the total number. As for peanuts, approximately 50 samples were taken, with the number of samples being 23 infected, or 46%. As for vegetable samples, only 7 samples were taken from onions, so the number of samples infected with the fungus was 3, meaning 42%. As for tomatoes, the number of samples taken was 20, while 15 infected samples were taken, i.e. 75%. As for sweet potatoes, it was The number of samples taken was 13, while the infected samples were 7, meaning a percentage of 53.8%. As for grapes, the number of samples taken was 23, while the infected samples were 19, meaning a percentage of 82.6%. As for watermelon, the number of samples taken was two, while the samples were infected with both samples, meaning a percentage of 100%. As for juices, they were a number The samples taken were only three samples, while the infected samples were one sample, meaning a percentage of 33.3%. As for milk, the number of samples taken was 7 samples, while the infected samples were one sample, meaning a percentage of 14.2%. Finally, bread, where the number of samples taken was 9 samples, while the number of infected samples was 4 samples, meaning a percentage of 44.4%. It was isolated and diagnosed based on some morphological characteristics.

**Table (1) shows Number of isolated samples**

| No | Sample | Number | Infected sample | Intact sample | Infection rate |
|---|---|---|---|---|---|
| **1** | Corn | 15 | 2 | 13 | % 13.3 |
| **2** | Peanut | 50 | 23 | 27 | % 46 |
| **3** | Onion | 7 | 3 | 4 | % 42 |
| **4** | Tomato | 20 | 15 | 5 | % 75 |
| **5** | Sweet potato | 13 | 7 | 6 | % 53.8 |
| **6** | grapes | 23 | 19 | 4 | % 82.6 |
| **7** | Watermelon | 2 | 2 | 0 | % 100 |
| **8** | Fruit juices | 3 | 1 | 2 | % 33.3 |
| **9** | milk | 7 | 1 | 6 | % 14.2 |
| **10** | Bread | 9 | 4 | 5 | % 44.4 |

The table below shows the type of fungi obtained from the different samples, Growth of the Aspergillus sp fungus appeared in samples taken from corn, peanuts, onions, juices, grapes, and milk,This type of fungus is capable of producing aflatoxin, and this is consistent with (El-Hamaky et al.,2023).While Fusarium sp fungi were obtained from tomato, sweet potato and watermelon samples. Fusarium sp. was found in tomatoes, sweet potatoes, and watermelon,Penicillium sp was found in Bread, as the most important characteristic of this type of fungus is that it produces several toxins such as Aflatoxin, Ochratoxins, Patulin, Oxalicacid, Malformin, Fumonisins, Zearalenone, as well as Penicillin.

**3.2-Types of fungi and the toxins they produce.**

**Table (2) shows some types of fungi and the toxins they produce**

| No | Sample | Mycotoxins secreting fungi | Mycotoxins |
|---|---|---|---|
| **1** | Corn | A. flavus | Aflatoxin |
| **2** | Peanut | Aspergillus sp | Aflatoxin |
| **3** | Onion | Aspergillus niger | Ochratoxins |
| **4** | Fruit juices | Aspergillus sp, Penicillium sp | Ochratoxins |
| **5** | Grapes | A.niger | Fumonisins |
| **6** | Milk | Aspergillus niger | Aflatoxin |
| **7** | Tomato | F. oxysporum | Zearalenone |
| **8** | Sweet potato | F. oxysporum | Zearalenone |
| **9** | Watermelon | Fusarium sp | Zearalenone |
| **10** | Bread | Penicillium sp | Penicillin |

**3.3- Using zinc oxide nanoparticles and testing their effect on some types of toxin-producing fungi.**

We are working on cultivating the fungi that were isolated from some samples and obtained in their pure form and then treating them with previously prepared nanoparticles and observing the effect of these particles on the growth of the fungus at different concentrations.

**Table (3) Shows zinc oxide nanoparticles and their effect on some harmful fungi**

| | A. flavus | A. niger | F. oxysporum | Penicillium sp |
|---|---|---|---|---|
| **Con.550 μg/mL** | 3.6 | 3.3 | 3.8 | 4.1 |
| **Con.600 μg/mL** | 3 | 2.9 | 2.8 | 3.6 |
| **Con.650 μg/mL** | 2.5 | 2.5 | 2 | 3 |
| **Con.700 μg/mL** | 1.8 | 1 | 0.9 | 2.1 |
| **Con.750 μg/mL** | 0.5 | 0.2 | 0 | 0.8 |
| **Con.800 μg/mL** | 0 | 0 | 0 | 0 |
| **tetracycline** | 1.9 | 1 | 0.5 | 0.7 |

The effect of zinc oxide nanoparticles on a few isolated toxin-producing fungi, such as A. flavus, is demonstrated by the study results in Table 3. The average diameter of the radial growth inhibition zone reached 3.6 when it was applied at a dose of 550 µg/ml. This prevented the development of mycotoxins, but to a small extent compared to the other concentrations. The average diameter of the radial growth inhibition zone reached three when used at a concentration of 600 µg/ml. The average diameter of the radial growth inhibition zone was 2.5 when applied at a concentration of 650 µg/ml. The average diameter of the radial growth inhibition zone was 1.8 at a concentration of 700 µg/ml, and 0.5 at a concentration of 750 µg/ml. The plates treated with the tetracycline antibiotic as a positive control had an average diameter of 1.9, while the zone of radial growth inhibition at a dose of 800 µg/ml had an average diameter of 0. Compared with the other concentrations and the tetracycline antibiotic, the average diameter of the radial growth inhibition zone for the concentration was 800.It is the best and largest in terms of inhibition.

When these concentrations were applied to the fungus A. niger, it became clear from the results of the study that the average diameter of the inhibition zones for radial growth reached (3.3, 2.9, 2.5,1 ,0.2, 0), respectively, when applied at the following doses (550, 600, 650,700, 750, 800) micrograms/ml, respectively, while when adding the tetracycline antibiotic to the media, the rate of inhibition of the fungus was 1. We note that at the concentration of 800, it was better and greater in terms of inhibition.

As for the F. oxysporum fungus, applying these concentrations we notice different results, as the zinc nanoparticles had a clear inhibitory effect on the F. oxysporum fungus. It was clear from the results of the study that the average diameter of the inhibition zones for radial growth reached (3.8, 2.8, 2, 0.9, 0.9, 0), respectively, when applied to the following doses (550, 600, 650, 700, 750) micrograms/ml on respectively, while when the antibiotic tetracycline was added to the medium, the fungal inhibition rate was 0.5 as a positive control. We note that at a concentration of 750, it was better and greater in terms of inhibition.

Finally, we apply and add these concentrations to the dishes that will be inoculated with the Penicillium sp fungus and monitor the inhibitory rates of the nanoparticles. The results of the study showed that the average diameter of the inhibition zones for radial growth reached (4.1, 3.6, 3, 2.1, 0.8, 0), respectively, when applied to the following doses (550, 600, 650, 700, 750, 800) micrograms/ml. Respectively, when adding the antibiotic tetracycline to the medium, the fungal inhibition rate was 0.7 as a positive control factor. We note that at a concentration of 800, it was better and greater in terms of inhibition and thus preventing the secretion of mycotoxins.

These results were compared with the results of dishes that were not treated with any concentration of antibody as a negative control agent, where the average diameter of the radial growth inhibition zone was 7.5.

## Conclusion

This study, as well as previous studies, propose the use of zinc oxide nanoparticles, which have antibacterial activity on several types of toxin-producing fungi, and which are considered a promising alternative to many antibiotics that have been proven to be resistant to organisms. Nanotechnology is also considered one of the most effective technologies in Combating bacteria and fungi. These are considered promising ways to prevent the growth of fungi and eliminate their harmful and dangerous effects.

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