



Efficiency of Nano powders of Cloves, Cinnamon, and Turmeric in Ochratoxin A (OTA) production by *Penicillium polonicum*

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Abstract

The study aimed to evaluate the efficiency of Nano powders of Cloves, Cinnamon, and Turmeric in inhibiting fungal growth and Ochratoxin a (OTA) production by *Penicillium polonicum* and reducing the toxin in liquid media. The research highlights the reduction of ochratoxin toxins using environmentally friendly plant Nano powders that are safe for human and animal consumption. They are mainly used in food as flavorings and preservatives. The Nano treatments of cloves, cinnamon, and the mixture (Cloves-Cinnamon-Turmeric) achieved complete inhibition of fungal growth and OTA toxin production by 100% for concentrations (2, 4, 6, 8%). The treatment of Nano Turmeric powder did not inhibit fungal growth at all concentrations. However, it inhibited OTA toxin production by 87 and 92% for concentrations 2 and 4% and by 100% for both concentrations 6 and 8%. In reducing OTA toxin, the mixture (Cloves-Cinnamon-Turmeric) was superior, with reduction rates of 95% for concentration 2% and 100% for concentrations 4, 6, and 8%. It was followed by the treatment of Clove Nano powder with reduction rates of 78, 84, 89, 94, and 100% for concentrations 0.8, 2, 4, 6 and 8% respectively. The treatment of Turmeric Nano powder came in third place in the efficiency of reducing OTA toxin with rates of 74, 79, 83, 87, and 100% for the same concentrations, respectively. As for Cinnamon Nano powder, it showed reduction rates of 68, 71, 79, 85 and 91% for the same concentrations, respectively.

Keyword: *Penicillium polonicum*, Ochratoxin A, Cloves, Cinnamon, Turmeric, Plant Nano powder

1.Introduction

Spices are a group of aromatic plants that have many uses in the food industry as preservatives. They are also used either as flavors and visual appeal for foods (1,2) or for medical purposes (3). One of the most important spices is *Syzygium aromaticum* (Clove). It is rich in active biological substances such as Eugenol, which is responsible for its aroma. Cloves have antioxidant, anti-glycation, antinociceptive and antimicrobial activities (4). In addition, Cloves contain other effective antimicrobial compounds such as Acetyl Eugenol, Beta-caryophyllen, Crategolic, Vanillin, and tannins (5, 6). Cinnamon (*Cinnamomum zeylanicum* C) contains tannins, phenols, and a volatile oil containing cinnamaldehyde and eugenol.



The biological activity of Turmeric (*Curcuma longa* L.) is mainly due to its curcumin content, which is 0.3–5.4% of turmeric, in addition to flavonoids, resins, and volatile oils (7). Ochratoxin A (OTA) is a mycotoxin that contaminates many different plant products and crops, including cereals, beer, wine, and dried fruits (8). It is produced by several species of *Aspergillus* and *Penicillium* (9).

OTA has been shown to be nephrotoxic, immunosuppressive, and carcinogenic (10). The International Agency for Research on Cancer (IARC) has classified OTA as a probable human carcinogen (Group 2B) (11). This study aimed to evaluate the efficiency of plant Nano powders in inhibiting fungal growth and reducing ochratoxin A in liquid media.

2. Materials and methods

1.2. *P.plonicum* fungal isolate

The molecularly characterized *P.plonicum* fungal strain registered in the NCBI Gene Bank under the serial number (LC843909) was active.

2.2. Preparation of Nano plant powders

The floral parts of cloves, cinnamon bark, and turmeric rhizomes were brought to the laboratory from local markets in Baghdad. They were ground in the Materials Laboratory / Ministry of Science and Technology using a large electric grinder type RS200 from Retsch Company, Germany, at a speed of 1500 rpm. Then, the samples were transferred to the sieve shaker to obtain a very fine powder. The grinding and sieving process was repeated. The powders were examined by an atomic force microscope (AFM) with specifications CoreAFM 2023, Nanosoft AG Switzerland.

2.3. Testing the ability of the fungal isolate *P.plonicum* to produce OTA toxin

Three glass flasks were taken, and 150 ml of liquid rice medium was added to each flask. Mix well on a magnetic stirrer and sterilize in an autoclave at 121°C and 1.5 kg/cm² pressure for 20 min. After cooling the medium to 45°C, tetracycline antibiotic 250 mg/L was added. Each flask was inoculated with five discs of 7-day-old fungal isolate using a cork piercer. Flasks were then incubated in a shaking incubator at 25 + 2°C for 14 days for OTA toxin production(13).

2.4. Extraction of OTA toxin

OTA toxin was extracted according to the method described in (14). The liquid medium was filtered using filter paper. The filtrate was transferred to a separating funnel with the addition of chloroform in the same volume as the filtrate. The funnel was shaken for 30 seconds while the expulsion of the formed gases was observed.

After the extract was separated into two layers, the lower layer containing chloroform was taken. The upper layer was neglected by passing it through Whatman No. 4 filter paper containing anhydrous sodium sulfate for the purpose of removing water. The filtrate was collected in a glass bottle, and its volume was determined and stored in the freezer until subsequent tests were conducted on it.

2.5. Determination of OTA poison using High-Performance Liquid Chromatography (HPLC) technology

HPLC model SYKAM (Germany). It was used to analyze and detection of Ochratoxin. The mobile phase was isocratic acetonitrile: D.W: formic acid (50:47:3) at a flow rate of 1.0 mL/min, column was C18-ODS (25cm*4.6 mm), and the detector Fluorescent (Ex = 365 nm, Em = 445 nm). The reduction rate of the poison was calculated according to the following equation (15):

$$\text{Toxin concentration in sample} = \frac{\text{Standard Toxin Concentration} \times \text{Sample Curve Area}}{\text{Standard Toxin Curve Area}} \times \frac{\text{Number of Dilutions}}{\text{Sample Volume or Weight}} \quad (1)$$

2.6. Testing the efficiency of clove, cinnamon, and turmeric nanopowders in inhibiting the growth of *P.polonicum* and preventing its production of ochratoxin A in a liquid rice medium.

The liquid rice medium prepared as in the previous paragraph was distributed in 250 ml flasks at a rate of 100 ml/flask. Then, the plant Nano powders (cloves T1, cinnamon T2, turmeric T3 and Clove-Cinnamon- Turmeric Nano powder mixture T4) were added at concentrations of (2, 4, 6, 8)%, with three replicates for each concentration for each treatment. Three flasks were left as a negative control treatment without addition.

The medium was homogenized using a magnetic stirrer. Then, the mouth of the flasks was closed with cotton and covered with aluminum foil. Then, they were sterilized in an autoclave at a temperature of 121 °C and a pressure of 1.5 kg / cm² for 20 minutes.

After the medium cooled to 45°C, the antibiotic tetracycline was added at a rate of 250 mg/L. Then, the flasks were inoculated with 5 mm diameter discs of the *P.polonicum* fungus isolate. They were incubated at a temperature of 25 + 2°C for 21 days (16). The OTA toxin was extracted and determined using HPLC.

2.7. Testing the efficiency of clove, cinnamon, and turmeric Nano powders in reducing OTA toxin in liquid rice medium.

Flasks containing sterilized liquid rice medium (100 ml) containing tetracycline antibiotic were inoculated with five 5 mm diameter discs of 7-day-old *P.polonicum* isolate using a cork piercer.

Flasks were incubated in a shaking incubator for 14 days to produce OTA toxin. After the incubation period, the fungus was killed in an autoclave at a temperature of 121 °C and a pressure of 1.5 kg/cm² for 20 minutes. Then the treatments (cloves T1, cinnamon T2, turmeric T3 and Clove-Cinnamon- Turmeric Nano powder mixture T4) were added at concentrations (0.8, 2, 4, 6, 8)% to all powders, with three replicates for each concentration for each treatment, leaving three flasks as a negative control treatment without addition. Then, the flasks were incubated again at a temperature of 25+2 °C for 21 days. OTA toxin was extracted and estimated by HPLC.

2.8. Statistical Analysis

The statistical program Statistical Analysis System (2012) -SAS was used to analyze the data to study the effect of different treatments on the studied characteristics according to a completely randomized design (CRD). The significant differences between the means were compared using the Least Significant Difference (LSD) test.

3.Results

3.1. Measurement of nanosize by AFM

The AFM showed that the particle size was Nano sized. The average sizes of the clove Nano powder particles ranged from 0-100 nm (**Figure 1**), for the cinnamon Nano powder 0-150 nm (**Figure 2**), and for the turmeric Nano powder 0-90 nm (**Figure 3**).

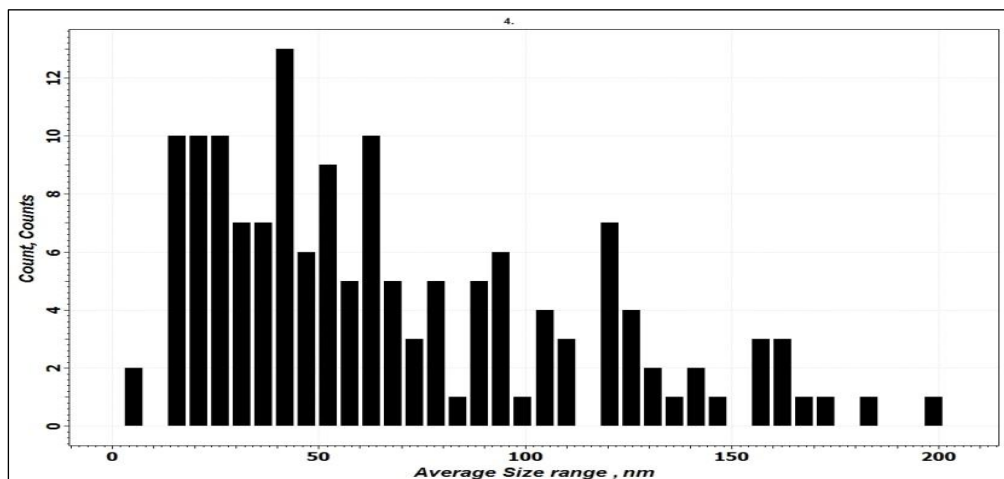


Figure1. Analysis of the size of the nanoparticles of the clove nanopowder using the AFM.

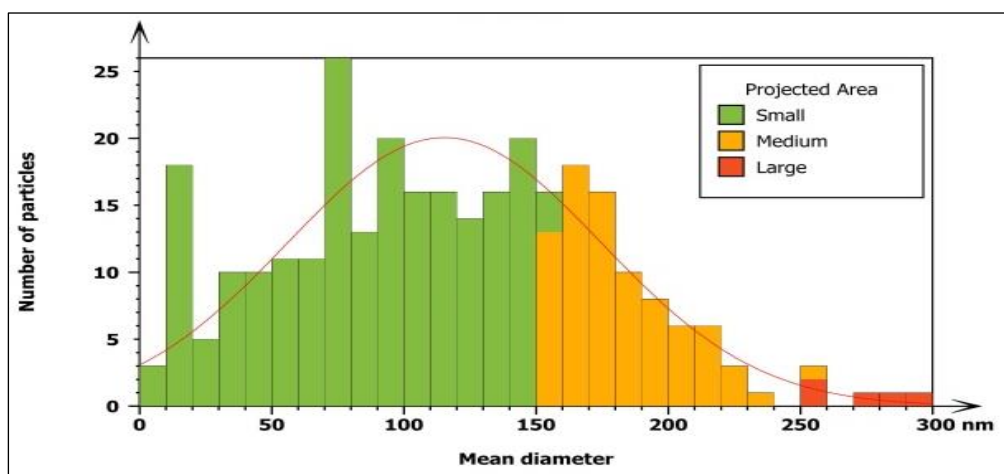


Figure2. Analysis of the size of the nanoparticles of the cinnamon nanopowder using the AFM.

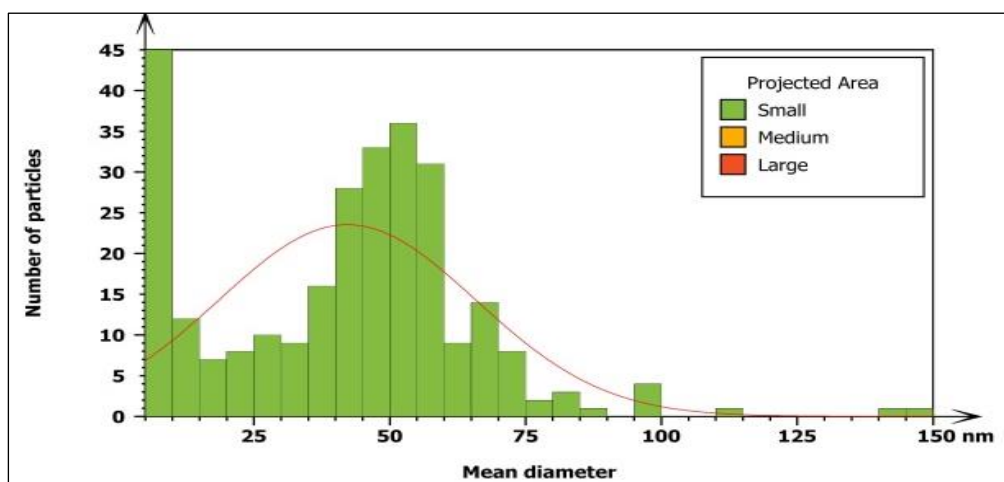


Figure3. Analysis of the size of the nanoparticles of the turmeric nanopowder using the AFM

3.2. Testing the ability of the fungal isolate *P. polonicum* to produce OTA toxin

HPLC technology showed the high ability of *P. polonicum* to produce OTA toxin. This is done by comparing the retention time of ochratoxin A produced by the fungus *P. polonicum* (Figure 4,B) with the retention time of the standard OTA toxin (Figure 4,A). Then, calculate the amount of toxin using the equation (1). The amount of toxin secreted by the fungus in the liquid medium reached 561.77ppb per gm.

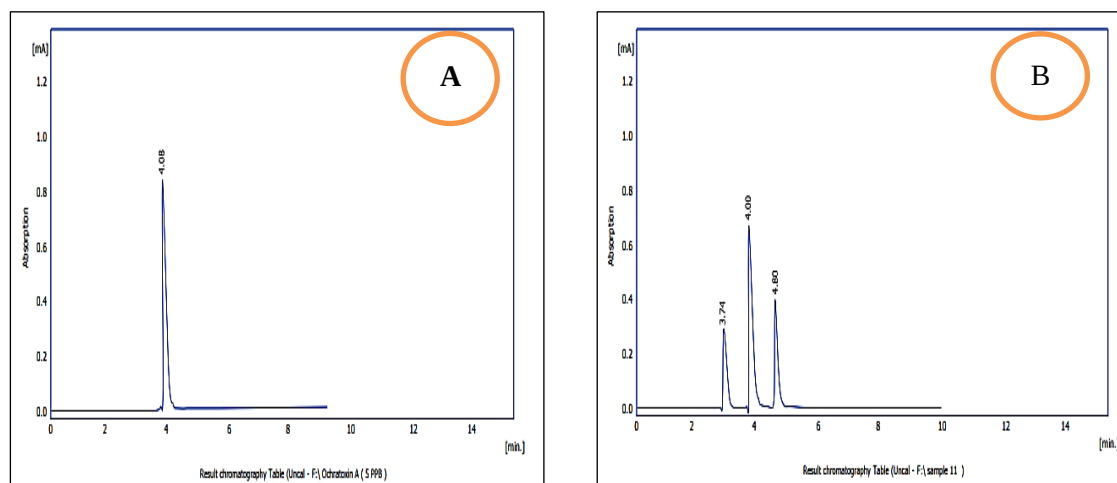


Figure4. Amount of ochratoxin A (OTA) using HPLC technology in the liquid medium in PPb. A.: standard model of ochratoxin A ; B: produced by the *P.polonicum* isolate

3.3. Inhibition of *P. polonicum* growth and prevention of its production of ochratoxin A in liquid rice medium using Nano powders of Cloves, Cinnamon, and Turmeric.

The results of high-performance liquid chromatography (HPLC) technology showed the ability of Nano powders of cloves, cinnamon, and (cloves- cinnamon –turmeric) treatments and all concentrations to inhibit *P. polonicum* and completely prevent its production of toxin by 100%. As for the Nano Turmeric treatment, it achieved inhibition of the growth of the fungus and inhibition of its production of toxin by 87 and 92% for concentrations 2 and 4%, respectively. Meanwhile, concentrations 6 and 8% gave 100% inhibition (**Table 1**).

Table 1. Effect of Nano powders of Cloves, Cinnamon, Turmeric and Clove-Cinnamon- Turmeric mixture on *P. polonicum* and inhibiting the production of ochratoxin A in liquid rice medium.

Treatment Name	Treatment concentration%	CON. (ppb)	Percentage%
T1	2	UDL	100
	4	UDL	100
	6	UDL	100
	8	UDL	100
T2	2	UDL	100
	4	UDL	100
	6	UDL	100
	8	UDL	100
T3	2	25.6	87
	4	14.9	92
	6	UDL	100
	8	UDL	100
T4	2	UDL	100
	4	UDL	100
	6	UDL	100
	8	UDL	100
Control		189.8	
L.S.D. value		---	7.348 *
(P≤0.05) *			

T1: Clove nanopowder, T2: Cinnamon nanopowder, T3: Turmeric nanopowder T4: Clove- cinnamon - turmeric nanopowder mixture.

3.4. Reduction of OTA toxin in liquid rice medium using Nano powders of Cloves, Cinnamon, and Turmeric

The results of HPLC technology showed high efficiency of the four Nano powders in reducing OTA toxin in a liquid medium. The results showed the superiority of treatment T4, then T1, then T3, and finally T2 for all concentrations, except for the 8% concentration in which treatments T1, T3, and T4 achieved a complete reduction of the toxin by 100%, outperforming treatment T2, which reduced the toxin by 91% (**Table 2**).

Table 2. . Effect of Nano powders of Cloves, Cinnamon, Turmeric and Clove-Cinnamon- Turmeric mixture in inhibiting the production of ochratoxin A in liquid rice medium.

Treatment Name	Treatment concentration%	CON. (ppb)	Percentage%
T1	0.8	41.8	78
	2	30.5	84
	4	22.9	89
	6	11.9	94
	8	UDL	100
T2	0.8	62.5	68
	2	56.9	71
	4	41.1	79
	6	28.9	85
	8	17.9	91
T3	0.8	50.2	74
	2	41.3	79
	4	33.1	83
	6	25.9	87
	8	UDL	100
T4	2	9.8	95
	4	UDL	100
	6	UDL	100
	8	*UDL	100
Control		193.6	
L.S.D. value		---	11.552 *

.(P≤0.05) *

T1: Clove nano powder, T2: Cinnamon nano powder, T3: Turmeric nan powder T4: Clove - Cinnamon - Turmeric nano powder mixture.

4. Discussion

Spices have active compounds with antifungal properties. These compounds include phenols, terpenes, saponins, and flavonoids (17,18). Among these compounds, specific compounds are mainly responsible for their antifungal activity: eugenol in cloves (19), cinnamaldehyde in cinnamon (20), and curcumin in turmeric (21).

The reason that leads to inhibition of the growth of *P. polonicum* in liquid rice medium and prevention of the production of ochratoxin A (OTA) by nanopowders of cloves, cinnamon, turmeric, and a mixture of (cloves- cinnamon -turmeric) is due to one or more of the following reasons: 1. Disruption of fungal membranes: The active compounds may form complexes with the fungal cell wall and soluble proteins inside the cell. This leads to the rupture of its membrane, the leakage of its contents, and then the death of the cell (22-24).

2. Generation of reactive oxygen species (ROS): These compounds may be a source of ROS, which react with nucleic acids and membrane proteins. This, in turn, inhibits the work of fungal enzymes (25). 3. Inhibition of mitochondrial function: Phenols and terpenes can inhibit the protein pump and ATPase enzyme in mitochondria (26). 4. Inactivation of cell enzymes:

The aromatic nucleus and phenolic OH group in phenols can form hydrogen bonds with the active sites of target enzymes (27).

All the nanopowders of the studied treatments except turmeric inhibited the growth of *P.polonicum*. They prevented the production of OTA toxin in liquid rice media. Interestingly, turmeric nanopowder did not show significant growth inhibition of the fungus despite significantly inhibiting the production of the toxin. This is consistent with what was reported by many previous studies. Studies have shown that some plant powders and their extracts can inhibit the production of mycotoxins without affecting the growth of fungi (28-30). Indicated (31) that *Fusarium oxysporium* and *P.digitatum* isolates were resistant to the essential oil of turmeric leaves. At the same time, *A.niger*, *A.flavus*, and *A.ficum* were more sensitive. The same is the case with what was mentioned by (32) that cinnamon powder at a concentration of 2% inhibited the production of aflatoxins by 97% in yeast and dextrose medium (YES), despite inhibiting the growth of *A.parasiticus* (strain NRRL 2999) by only 31%. The variation in the effectiveness of turmeric is due to its possession of various compounds, some of which may be inhibitory, some of which may promote growth, and others have no effect (33). In addition, the presence of active compounds with each other may cause a negative effect rather than an effective one (34). The treatment of the nanopowder of the mixture (cloves-cinnamon-turmeric) showed superiority over all nanopowders. This is due to the synergistic action of the active compounds, especially Cinnamaldehyde, eugenol, and Curcumin. The presence of different concentrations of these compounds may lead to enhancing the antifungal effect (35). Since the presence of combined essential oils is more effective than the separate compounds (36). These spices, their extracts, and essential oils have been widely used to inhibit bacteria and fungi (37-42). Extracts and powders of several medicinal plants have been shown to inhibit the growth of *A. flavus* and aflatoxin production (43-45). In addition, nanoparticles, such as silver nanoparticles (AgNPs) synthesized by green synthesis (46-48), and Nano metal oxides synthesized with eugenol and nanozinc oxide, have shown anti-candida, anti-bacterial, and anti-filamentous fungal activity (49-51). The effectiveness of the nanopowders under study in reducing OTA toxin can be attributed to their high surface area, which contributes to the adsorption of the toxin, or the ability of some of their compounds to bind to OTA and decompose it into a non-toxic compound (52,39). This is done through the interaction of hydroxyl groups in phenolic compounds with the active groups in the toxin molecule (53). It was found that eugenol contains allyl (-CH₂-CH=CH₂), phenol (-OH), and methoxy (-OCH₃), which may interact with the active sites in OTA. In the same context, (54) indicated that the use of a nanopowder of *Ganoderma lucidum* fungus at a concentration of 5 mg reduced aflatoxin B₁ (AFB₁) by 27.63% in YES medium and by 22.17 - 88.64% in corn grains contaminated with the toxin when the concentration of nanopowder increased from 5 - 1000 mg, respectively. This is consistent with the results of this study, which concluded that higher concentrations of plant nanopowders led to a greater reduction in OTA toxin. Plant powders and extracts are a rich source of bioactive compounds (such as flavonoids, tannins, and alkaloids) that possess antimicrobial properties (55).

5.Conclusion

Plant Nano powders are safe for human and animal consumption. They are an alternative to nanoparticles made from some metals that have long-term health effects. Plant nanoparticles also have the same properties as manufactured nanoparticles in terms of small size and high surface area. They also have active compounds that contribute to preserving

food from fungal contamination and the production of mycotoxins. These materials can also provide a promising solution in resisting antibiotics and combating pathogens.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Ethical Clearance

This study was conducted after obtaining the approval of the Scientific Research Ethics Committee at the College of Education for Pure Sciences Ibn Al-Haitham, University of Baghdad (EC-59) on December 24, 2024. This procedure is in line with the guidelines set forth by the Iraqi Ministry of Health and Environment.

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