Evaluation Efficiency of Silver Nanoparticles Enriched by Honey in the Detoxification of Aflatoxin B1

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Abstract

This study was carried out at mycotoxins laboratory, plant protection department, College of agriculture, University of Baghdad to test of efficiency of silver nanoparticles, silver nanoparticles enriched by honey and honey in *Aspergillus flavus* fungus inhibition and prevent of aflatoxin B1 toxin production or reduction aflatoxin toxin in the stored grains of maize.

The results of this test showed the efficiency of the honey enriched by silver nanoparticles, the honey and silver nanoparticles in inhibition of *Aspergillus flavus* fungus in the culture PDA after subjection the prepared silver nanoparticles to test by zeta potential device to measure sizes of the silver nanoparticles that were between 25 - 40 nm. It is noticed that using the honey enriched by silver nanoparticles, the honey and silver nanoparticles treatments in 2,4 and 6% concentrations respectively caused inhibition of the *Aspergillus flavus* fungus at rates of (62%, 70% and 83%), (59%, 64% and 73%) and (56%, 61%, 66%) respectively. The obtained results by using the high performance liquid chromatography device (HPLC) confirmed that the honey enriched by silver nanoparticles that added to the stored maize grains reduced the aflatoxin B1 toxin by 88% rate compared with the honey and silver nanoparticles treatments in which the reduction rates were 33% and 66% respectively.

Keywords: Honey; AG NP; Aflatoxin B1; Aspergillus flavas; Detoxification and Inhibition

Introduction

The aflatoxin toxins are considered as the more important and dangerous of the mycotoxins that may pollute the herbages and foods and play main role in cancer cases occurrence such as liver cancer in human and animal and the severe chronic toxification and genetic mutations and malformations besides their biological effects in the digestive and urine tract, blood vessels, kidneys and neurological disorders [1].

Safety of foods and herbages and absence of the mycotoxins in them are considered of the necessary things that must pay an attention. Some of the international organizations such as food and drugs U.S. administration (U.S.FDA) limited the safe concentration of aflatoxin in the maize for human and animal consumption must not be above 20 PPb in order to decrease risks of exposure to such toxins [2], besides doing many studies on destruction and remove of the mycotoxins in foods and herbages, these studies varied among the chemical, physical and biological methods [3].

Honey is considered as a natural material that has an active role in inhibition of a lot of microorganism due to presence of many active compounds such as phenolic acids, flavonoids, glycosides and alkaloids and others [4]. Some of the studies showed that the inhibition activity of the microorganisms may be also due to the low PH of the honey [5]. This research includes application of the nano technique in food and agriculture field by using the silver nanoparticles enriched by honey in destruction the aflatoxin toxin, the new studies indicated

that the particles of nano mineral oxides have special physical and chemical properties due to their limited sizes and high densities and have surface positions to react compatible with the biological systems and this allows to bind them with biological compounds [6,7]. So, the present research possibility of using the silver nanoparticles enriched by honey which are considered friend of the environment and safe to human and animal [8] and the possibility of using honey in destruction of aflatoxin B1 toxin due to its high efficiency in inhibition growth of the fungus that produces aflatoxin B1 toxins were investigated.

Materials and Methods

The laboratory experiment

The isolation of the *Aspergillus flavus* fungus that produces aflatoxin B1 toxin obtained from the mycotoxins laboratory, plant protection Department, College of Agriculture, by help of the assistant prof. Halima Z. Hussein, the isolation was genetically recognized (partially by (PCR) technique).

Growth of the isolation on rice medium

The medium was prepared according to Shotwell., *et al*. [9] by adding 150 gram of rice to 100 ml distilled water and then it was put into large size (20 cm diameter and 5 cm height) petri dish and then it was sterilized inside autoclave and then the sterilization repeated again after 24 hours from the first sterilization to insure killing all the organisms. The mediums were smeared by the *Aspergillus flavus* fungus isolation using five nipping which were taken by using cork auger for each petridish (3 petridish were used), then all the petri dishes were incubated at $25^{o}C\pm 2$ and shaken for 7 days and were left for 21 days for toxin production.

Aflatoxin B1 toxin extraction

The toxin was extracted by using method of Blazer 1978 [10], this method is highly sensitivity and efficiency. Twenty five grams sample of the growing fungi on the rice media was weighted after drying and grinding and then was put in glassy flasks and 100 ml of the extraction solution, which consisted from aceto nitrile and water at ratio of 90:10, was added to each flask and the nozzle of the flaks strongly closed. Flasks shaken process was used to mix the flask content for 30 minutes, the solution was filtered through filtration flask using double rings (12.5 cm) filter paper and the extraction was put in separator funnel (500 ml capacity) and 25 ml of hexan was added to get rid of fats (defatting) and the funnel was shaken gently for 30 seconds with allowing to the produced gases to leave as it is required (two times at least) and the funnel was kept on the stand for one minute in order to separate the two layers. The upper layer was ignored and this operation was repeated three times to insure that all the fats were got rid. To the lower layer, 25 ml of distilled water and 8 ml of saturated $NaHCO_3$ and 25 ml chloroform were added and after three minutes two separated layers (upper and lower layers) were got and the upper layer was taken. This operation was repeated two times. The extracted solution was transferred to separator funnel (500 ml capacity) and 7.5 ml of concentrated HCL and 10 ml chloroform were added and it was extracted again, the two low layers were collected together and passed through the double rings (12.5 cm) filter paper contained layer of the non-hydrated Na_2SO_4 to get rid of the remain water, the extraction was evaporated by using the rotary evaporator at $70^{o}C$ until drying or by using oven at 50oC, then the precipitate was dissolved in 1 ml of mixture of aceto nitrile: benzene at rate of 2:98 and the solution was put into small tubes (vials) and kept freeze until the test time.

Determination of toxin by HPLC instrument

The total amount of the aflatoxin B1 toxin in the rice medium was determined in department of food and biotechnology, agricultural research center, sciences and technology Ministry by using the high performance liquid chromatography (HPLC), model LC-2010 H1, shimadzu co. and a separation column type (c18-54 CN) reverse phase phenomenex (250 mm × 4.6 mm) and mobile phase consists of mixture of 180 ml aceto nitrile and 820ml distilled water and 10 ml glacial acetic acid and they were mixed by the mixer before test to get rid of the produced gases, the flow rate was 0.5 ml per minute and their ultra violet rays absorption was followed at 365 nm wave length.

The toxin concentration in the samples was calculated by using the followed equation:
Conc. of toxin in the sample= (curve area of the sample/the standard toxin curve area) × concentration of the standard toxin × dilution number

Test of the efficiency of the silver nano particles enriched with honey in inhibition of *Aspergillus flavus* laboratory

For setting the laboratory experiment, three treatments were tested (silver nano particles only) which was obtained from the American company (MTI) and its properties were as follow.

Silver (Ag)	Nano powder
Purity	99.99+%
Aps (by TEM)	40 - 50 nm
SSA	5 - 10 m^2/g
Morphology	Spherical
Bulk Density	1.2 - 1.3 g/cm^3
True Density	10.5 g/cm^3

Three concentrations of silver nanoparticles 2, 4 and 6% were added to the culture media to test its efficiency in fungus inhibition and the second was the honey enriched by silver nanoparticles which was done by taking the same concentrations 2, 4 and 6% of the silver nanoparticles added to the honey and then added to the culture media PDA, and the third was using honey only that was done by taking the same concentrations 2, 4 and 6% to the culture media PDA beside presence of the control (free of honey and silver nanoparticles materials). All the treatments were subjected to the ultrasonic device for eight minutes in laboratories of biotechnologies research center, Al- Nahrain University, then size of the silver nanoparticles was measured by zeta potential instrument at laboratories of environment and water center/Ministry of sciences and technology. The cultures which were treated with the honey, honey + silver nanoparticles and silver nanoparticles materials were poured in petridishes using three replicates for each treatment and smeared by *Aspergillus flavus* fungus. The dishes were incubated in an incubator at 25 ± 2°C and then the inhibition rate was calculated by estimating a average of the vertical columns according to the following equation.

Inhibitor percentage: (colony diameter medium of control treatment - colony diameter medium of treatment/colony diameter medium of control treatment) × 100

The storage experiment

It was done in mycotoxins laboratory/College of agriculture/University of Baghdad and maize seeds were got from Mabeen Al-Nahrain company, Ministry of agriculture. The seeds were contaminated by the toxin producer isolation that was grown on rice medium at 4 gram per kilogram rate after mixing well with 5 ml of water to insure mixing with maize seeds. The mixing operation lasted for 5 days till getting 16% moisture content in special dissectors at three replicates for each treatment using one kilogram for each replicate with leaving three replicate without treating for comparison, then they treated and left for 5 days. After the maize seeds were ready and contaminated with toxin, they sterilized in an autoclave at 121°C, 1.5 kg cm^{-2} pressure for 20 minutes and then they treated with silver nanoparticles, silver nanoparticles enriched by honey and honey at 6% concentration for each treatment using spraying for each treatment and the control treatment was treated with water only for comparison. The treatments were stored at laboratory temperature for one month and then were dried in an electrical oven and twenty five of the contaminated maize seeds were grinded for aflatoxin B1 toxin extraction and determination of its concentration by HPLC device for calculating toxin reduction rate using the following equation.

Reduction percentage = (concentration of toxin in control treatment - sample concentration/concentration of toxin in control) × 100

Results

The quantities determination of AFBI toxin by using HPLC

The results showed that the *Aspergillus flavus* fungus isolation was productive to AFBI toxin at 10 microgram. g^{-1} and this agrees with finding of last studies which showed that the ability of *Aspergillus flavus* fungus to produce AFBI toxin in high concentrations that may reach 18.6 - 740 ppm [11,12].

Test of efficiency of the silver nanoparticle enriched with honey, silver nanoparticles and honey in inhibition of *Aspergillus flavus* fungus in the culture medium PDA laboratory

After examining of the silver nanoparticles by Zeta potential device to measure size of the silver nanoparticles and select the suitable size for doing the experiment, it was found that the sliver nanoparticles size ranged 25 - 40 nm (Figure 1 and 2).
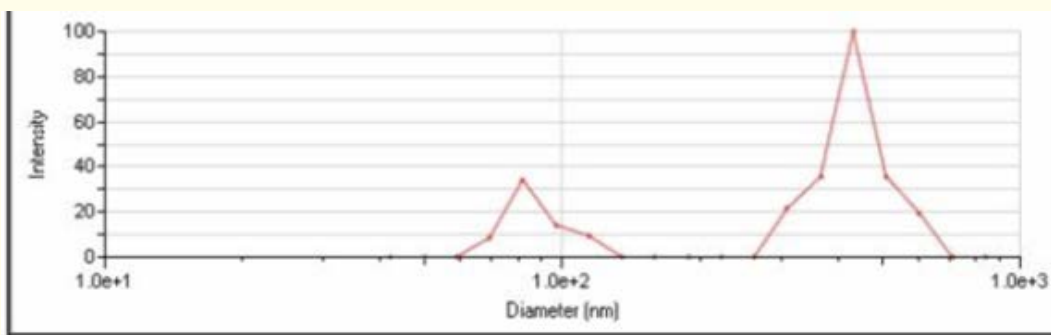

Figure 1: *Results of test of size of the sample particles by zeta potential device.*
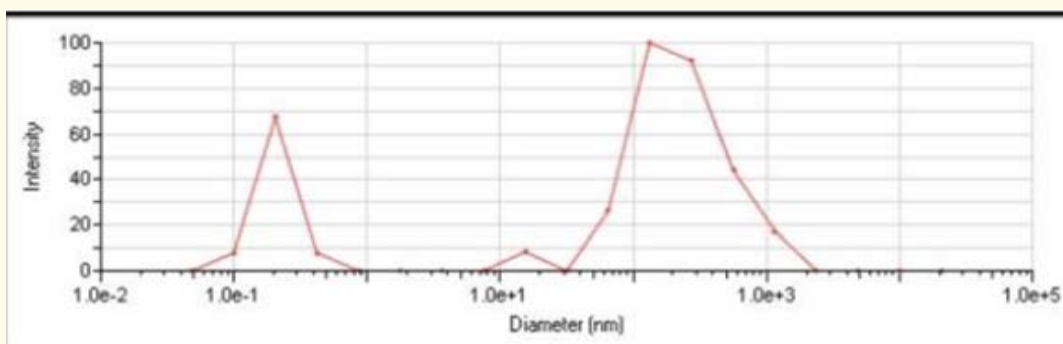

Figure 2: *Results of test of size of the sample particles by zeta potential device.*

Test of efficiency of different concentrations of the silver nano particles enriched by honey in inhibition of *Aspergillus flavus* fungus in the culture medium PDA laboratory

Results of table 1 shows efficiency of the silver nanoparticles enriched by honey in limiting growth of the *Aspergillus flavus* fungus. The 6% concentration was the more efficiency with clear significant differences besides presence of significant differences between the three concentrations and control treatment.

Concentrations	R1	R2	R3	Average
2	0.62	0.59	0.65	0.62b
4	0.65	0.67	0.77	0.70b
6	0.79	0.82	0.87	0.83a
0	0.00	0.00	0.00	0.00c
LSD 0.05				0.08

Table 1: *Test of efficiency of different concentrations of the silver nano particles enriched with honey in inhibition of the Aspergillus F. fungus grown on PDA medium.*

Test of efficiency of the silver nano particles in inhibition of *Aspergillus flavus* fungus in the culture medium PDA laboratory

Results of table 2 show presence of clear significant differences between the concentrations and control treatments and the 6% concentration was superior in inhibition of *Aspergillus flavus* fungus in significant differences, this results agree with many studies that referred to efficiency of silver nano particles in inhibition of many fungi and agree with study of Hussein and Abdul-Hasan [13] who emphasized to efficiency of silver nanoparticles in inhibition growth of *Fusarium solani* fungus and with Abdulrahaman and Hussein [14] study about inhibition of *Aspergillus flavus*.

Concentrations	R1	R2	R3	Average
2	0.56	0.61	0.59	0.59b
4	0.62	0.60	0.71	0.64b
6	0.72	0.71	0.76	0.73a
0	0.00	00.0	0.00	0.00c
LSD 0.05				0.08

Table 2: Test of efficiency of silver nano particles concentrations in inhibition of Aspergillus flavus grown on PAD medium.

Test of efficiency of honey in inhibition of *Aspergillus flavus* in the culture medium PDA laboratory

The results in table 3 show presence of non-significant differences between the used concentrations in inhibition of *Aspergillus flavus* with presence significant differences between the concentrations and the control treatments, these results agree with many studies that referred to honey efficiency in limiting growth of bacteria and some funguses [15].

Test of efficiency of the silver nanoparticles enriched with honey, silver nanoparticles and honey in reduction of the aflatoxin B1 under storage condition

The results in table 4 show the high ability of the silver nanoparticles enriched with honey in reduction of the aflatoxin B1 toxin and the reduction rate reached 88.88% compared with the rest treatments that were 66.66% and 33.33% for silver nano particles and honey treatments respectively, these results agree with many studies that mentioned to efficiency of silver nanoparticles in reduction of aflatox-inB1 toxin and to the role of the medium in toxin reduction, that was reported by puzyer., *et al*. [6] to possibility of use the nanoparticles in adsorption AFBI toxin and also by Gibson., *et al*. [16] who mentioned to the efficiency of nanoparticles in toxin reduction.

Treatments	Concentration of Aflatoxin B1 Microgram g^{-1}	Reduction Rate
Control	0.09	0.00a
Silver Nanoparticles	0.03	66.66Bc
Silver Nanoparticles Enriched By Honey	0.01	88.88c
Honey only	0.06	33.33ab
LSD0.05		0.03

Table 4: Test of efficiency of the silver nanoparticles enriched with honey , silver nanoparticles and honey in reduction of aflatoxin B1 toxin under storage condition.

Discussion

The obtained results showed the activity and the high efficiency of honey enriched by silver nanoparticles and silver nanoparticles only and this results agree with many studies that proved efficiency of silver nanoparticles as founding of Kim., *et al.* (2012) that use of

100 ppm of silver nanoparticles caused inhibition of pathogenic fungal causes that cause plant diseases on PDA medium. A new study using an experiment out the living body that use of silver nanoparticles at 100 ppm in case loaded on Graphic oxide had big effect in inhibition of *Xanthomonas* performance that causes bacterial spots in tomatoes grown in glass houses [17].

The results showed efficiency of honey enriched by silver nanoparticles and the silver nanoparticles in reduction of aflatoxin B1 toxin at 88.88 and 66.66% rates respectively. The variance reason in the reduction rate in the tested treatments may be due to kind of the used material in the test which may be differ of each material in the test with the toxin and adsorbing it and they caused getting significant differences in the adsorbed toxin concentration level between the tested treatments and the control treatment and these agree with Othman., *et al.* [12] finding that adding the silver nanoparticles caused reduction in mycotoxins production in 81.1 - 95.5% rate and work of the silver nanoparticles may change mold metabolism operation and using high concentration of silver nanoparticles reflected clear effect at 81 - 96% rate and reduce mold cell toxicity at 50 - 75% rate [18].

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