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Honey mediated silver nanoparticles and their inhibitory effect on aflatoxins and ochratoxin A

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ABSTRACT

Silver nanoparticles (AgNPs) have potential antimicrobial activity against bacteria and fungi. The synthesis of AgNPs have been reported using several chemical and physical methods which are not friendly environment. Therefore, our technique has focused on the synthesis of AgNPs by natural compounds. The aim of this study has been to synthesis AgNPs by safe nontoxic method using Egyptian honey (EH) as reducing and capping agents and to investigate its ability to reduce the mycelial growth and the production of aflatoxins (AFs) and ochratoxin A (OTA) by *Aspergillus flavus* and *Aspergillus ochraceus*, respectively. AgNPs have been characterized by UV-Visible Spectrophotometer, Dynamic Light Scattering (DLS), Fourier Transform Infrared Spectroscopy (FTIR), and Transmission Electron Microscope (TEM).The obtained results indicated that the synthesis of honey AgNPs depends on the concentration of bulk metal ($AgNO_3$) used in the synthesis process. The TEM image has revealed the formation of spherical well dispersed AgNPs, while the main size of AgNPs detected by DSL is 9.9 nm. Our results have indicated that 3 mg $^{-100\ ml}$ media of honey derived AgNPs have reduced the aflatoxin (AF) G_1, G_2, B_1 andB_2 production by *A. parasiticus* to 77.55, , 62.91, 58.76 and 66.56%, respectively and ochratoxin A (OTA) by *A. ochraceus* to 79.85 % with significantly inhibitory effect on mycelial growth. The percentage of reduction depends on the AgNPs concentration.

INTRODUCTION

In the recent years, silver nanoparticles (AgNPs) have attracted much attention due to their widely applications in different field such as, medicine, biotechnology, optics, microelectronics, catalysis, information storage and energy conversion (Ghaseminezhada *et al.*, 2012). The synthesis of AgNPs has been reported using various methods including physical and chemical methods, electrochemical reduction and photochemical reduction. These methods focused on the use of a large amount of toxic materials with high temperature conditions. Therefore, the recent researches have been directed to us the green chemistry, as a simple, clean, environmental-friendly, cheapest and safe methods, for biosynthesis of nanoparticles (Narayanan and Sakthivel, 2010). Green synthesis of AgNPs should involve three main steps including: selection of solvent medium, selection of environmentally agreeable reducing agent, and selection of nontoxic substances for the silver nanoparticles stability. Asmathunisha and Kathiresan (2013) reported that the synthesis of nanoparticles may be triggered by several compounds such as carbonyl groups, terpenoids, phenolics, flavonones, amines, amides, proteins, pigments, alkaloids and other reducing agents presenting in the plant extracts and microbial cells. Honey is a natural food produced by bees from nectar or secretion of flowers. Honey has a content of 80-85 % carbohydrates, 15-17 % water, 0.3 % proteins, 0.2 % ashes, and minor quantities of amino-acids and vitamins as well as other components in low levels of concentration (White, 1975).

In addition the use of honey in the synthesis of AgNPs has been reported recently (Philip, 2010; Sreelakshmi *et al.*, 2011; Obota *et al.*, 2013). Many mycotoxigenic fungi can contaminate food and feed stuff and produce their secondary toxic metabolites.

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The most important mycotoxinsfor human and animal health are aflatoxins-AF (B_1, B_2, G_1, G_2), ochratoxin A (OTA), fumonisins (FB1, FB2), zearalenone (ZEA) and trichothecenes (deoxynivalenol-DON, T-2, HT-2). Aflatoxins (AFs) are produced by different fungi, particularly *Aspergillus flavus* and *Aspergillus parasiticus* (El-Desouky *et al.,* 2013). The four major classes of AFs, are B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2), which are each distinguished by their fluorescence color under ultraviolet light (B, blue; G, green). The International Agency for Research on Cancer (IARC) has classified AFB_1, AFB_2, AFG_1 and AFG_2 in the group I and OTA in group 2B as human carcinogen (IARC, 2002).AFs and OTAare known to be hepatocarcinogenic, mutagenic, teratogenic and immunosuppressive in both animals and human Pfohl-Leszkowicz *et al.,* 2007; Wu and Khlangwiset, 2010). The control of these mycotoxins is important since their occurrence in foods and feeds in continuously posing threat to both health and economics all over the world (Basappa and Shantha, 1996).Various workers have used different methods of detoxification Tocontrol the production of these mycotoxins in the feed and food stuff and these methods resulting in varying degree of success (Kasmani *et al.,* 2012; Hussain *et al.,* 2012). Recently, different substances have been used for reduction of the mycotoxins from the contaminated feed to protect animals against the harmful effects of them. Various natural and synthetic agents are known to prevent both mycotoxigenic mould growth and mycotoxin formation (Mahoney *et al.,* 2010).

In recent years, nanoparticle (NP) materials have received increasing attention due to their unique physical and chemical properties, which differ significantly from their conventional counterparts (Stoimenov *et al.,* 2002). The recent advancements in the field of nanotechnology have made AgNPs to be widely used as a novel therapeutic agent as antibacterial, antifungal, antiviral, anti-inflammatory and anti-cancerous agents (Otari *et al.,* 2015). On the other hand stable colloidal solutions of AgNPs were found to be effective against *Aspergillus* and *Penicillium* (Gajbhiye *et al.,* 2009). The inhibitory effect of metal nanoparticles on growth and production of mycotoxins by mycotoxigenic fungi was previously studied by Yehia and Ahmed (2013). They recorded that ZnO nanoparticles control the production of the mycotoxins fusaric acid and patulin produced by *F. oxysporum* and *P. expansum* (respectively) in concentration dependent manner. There are no previous studies on the effect of honey AgNPs on the fungal production of AFs and OTA. Therefore, the aim of this study has been to synthesis AgNPs by ecofriendly method using Egyptian honeyand to study their inhibitory effect on AFs and OTA production by *Aspergillus parasiticus* and *Aspergillus ochraceus*, respectively

MATERIALS AND METHODS

Material

Egyptian honey (EH) used in this study was obtained from the faculty of Agriculture, University of Zagazig, Egypt.

Fungal strains

The fungal strains used in this study were *Aspergillus parasiticus* (ATCC 185592) and *Aspergillus ochraceus* (ATCC 22947), obtained from MIRCEN, (Microbial Research Center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Chemicals and solvents

Silver nitrate, yeast extract and sodium sulphate anhydrous were obtained from Sigma-Aldrich, France. AFs (mix) and OTA standards were purchased from Sigma, chemical Co. (St. Louis, MO, U.S.A). Stock solutions and standards were prepared and assayed according to AOAC Method 971.22, 2005). All solvents were of HPLC grade. The water was double distilled with Millipore water purification system (Bedford, M A, USA).

Methods

Synthesis of silver nanoparticles

For the reduction of silver ions, 5 ml of 10% EH was added to 5 ml of different concentration of aqueous $AgNO_3$ (1, 10, 20, 30, 40 and 50 mM). The reaction mixture was stirred properly and incubated at 30 ^{0}C for 72 h. The solution turned to yellowish-brown indicating the formation of silver nanoparticles. The AgNPs were collected by ultracentrifugation, then re-dispersed in sterilized bi-distilled water and collected again by ultracentrifugation. This procedure was repeated three times. The AgNPs were air dried and a definite weight was re-dispersed in sterilized double distilled water by ultra-sonication as a stock solution. The stock solution was diluted with sterilized bi-distilled water to prepare the final concentrations used for antifungal study.

Characterization of AgNPs

UV–visible spectral analysis

The reduction of silver ions was routinely monitored by visual inspection of the solution. The color changes of the reaction mixtures to dark- brown are evidence for the formation of silver nanoparticles (AgNPs). 3-mL samples were withdrawn at various intervals from each $AgNO_3$ concentration, and the absorbance was measured by a double beam UV–visible spectrophotometer using Lambda 2 spectrophotometer Perkin-Elmerat a resolution of 1 nm in the range 200–600 nm.

Dynamic light scattering (DLS)

The size distribution and average size of the synthesized AgNPs were determined by dynamic light scattering (DLS). DLS (Malvern, UK) measurements were carried out for size ranges from 0.1 nm to 10 mm.

Fourier-transform infrared spectroscopy (FTIR)

The characterization of functional groups on the surface of AgNPs was performed by Fourier-transform infrared spectroscopy (FTIR 6100, Perkin-Elmer, Germany), and the spectra were scanned in the 400–4000 cm^{-1} range at a resolution of 4 cm.

Transmission electron microscopy (TEM)

The morphology of the synthesized AgNPs was observed by a Jeol JEM-1400 transmission electron microscope (TEM). TEM sample was prepared by drop-casting a dispersion of AgNPs on carbon-coated copper grids, which were allowed to dry at room temperature.

Assay of antifungal activity

The antifungal activity of synthesized AgNPs by EH was tested on the mycotoxigenic strains, *A. ochraceus* and *A. parasiticus* using agar well diffusion technique. Spores suspension of these fungi was prepared and adjusted to be approximately (1×10^6 spores^{-ml}). 1 ml of spore suspension was inoculated into each plate containing 25 ml of sterile PDA medium. Wells of 5 mm diameter were made on the PDA surface and filled with the gradual concentrations of (10, 20, 30 and 40 µg) of AgNPs colloids. The plates were incubated at 28 ºC for 72 hrs. PDA medium with the same amount of spores, without nanosilver, was used as a control sample. After incubation time, the plates were tested for the mycelial growth inhibitory zones around the wells.

Evaluation the effect of EH AgNPs on the production of AFs and OTA in liquid mediummedium.

The yeast extract sucrose (YES) culture medium (2% yeast extract and 15% sucrose) was used for AFs and OTA production according to the method of El-Desouky *et al.* (2012). The culture medium was poured into 250ml Erlenmeyer flask, and autoclaved at 121 ºC for 15 min, cooled at room temperature and inoculated with approximately (1×10^7) spores suspension of *Aspergillus parasiticus* and *Aspergillus ochraceus* both separately. Gradual concentrations of AgNPs (1, 2 and 3 mgAgNPs$^{-100\ mL}$ media) were added to the YES broth and incubated at 28 ºC for 14 days. After the end of incubation period, the mycotoxins (AFs and OTA) were estimated.

Extraction and determination of AFs

AFs were extracted according to the method described by El-Banna *et al.*, (1987). Extraction was carried out using 20 ml of chloroform (twice with 10 ml media), and homogenization for 3 min in a separation funnel. The chloroform phase was filtered through filter paper Whatman No. 3 and concentrated to dryness under a nitrogen stream.

Determination of AFs by HPLC

Derivatization: The derivatives of samples and standard were done as follow: 100 µl of trifluoroacetic acid (TFA) were added to samples and mixed well for 30 s and the mixture stand for 15 min. 900 µl of water acetonitrile (9:1 v/v) were added and mixed well by vortex for 30 s. The prepared mixture was used for HPLC analysis. The HPLC system consists of Waters Binary Pump Model 1525, a Model Waters 1500 Rheodyne manual injector, a Watres 2475 Multi- Wavelength Fluorescence Detector, and a data workstation with software Breeze 2. Aphenomenex C_{18} (250 x 4.6 mmi.d.), 5 µm from Waters corporation (USA). An isocratic system with water: methanol: acetonitrile (240:120:40v/v/v) was used. The separation was performed at ambient temperature at a flow rate of 1.0 ml/min. The injection volume was 20 µl for both standard solutions and sample extracts. The fluorescence detector was operated at wavelength of 360 nm for excitation and 440 nm for emission. Concentrations of AFs in samples were determined from the standard curve using peak area for quantitation.

Extraction and determination of OTA

Ten ml of culture medium were filtered through 0.2 µm syringe filter, and extracted with 20 ml of chloroform. The chloroform phase was filtered through filter paper Whatman No.3 with sodium sulphate anhydrous and concentrated to dryness under a nitrogen stream. The precipitate was dissolved in 1mL water: acetonitrile (3:1 v/v) and mixed well by vortex for 30 s. This mixture was used for HPLC analysis. An isocratic system with acetonitrile: water: acetic acid (55:43:2) was used. The separation was performed at ambient temperature at a flow rate of 1.0 ml^{-min}. The injection volume was 20 µl for both standard solutions and sample extracts. The fluorescence detector was operated at wavelength of 335 nm for excitation and 465 nm for emission. OTA concentrations in samples were determined from the standard curve using peak area for quantitation

Statistical analysis

All data were statistically analyzed using the General Linear Model procedure of the SPSS var.18. The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio. All statements of significance were based on probability of $P < 0.05$.

RESULTS

UV-Visible spectroscopy

The formation of AgNPs using different concentration of $AgNO_3$ (1-50 mM) and 10%final concentration of local Egyptian honey was confirmed by UV-Visible Spectroscopy analysis in the range of 200-600 nm. The current results have indicated that the addition of 5 ml of different concentrations of aqueous $AgNO_3$ (1, 10, 20, , 30, 40 and 50 mM) to 5 ml of 10% EH resulted in formation of brown solution in the reaction mixture containing (20, 30, 40 and 50 mM $AgNO_3$) after 72h of incubation at 30 °C (Fig. 1).

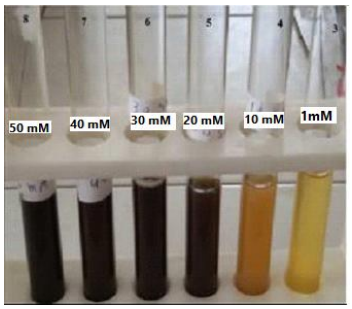

Fig 1: Color change of $AgNO_3$ at different concentrations (1-50 mM) mixed with 10% EH after 72 h of incubation.

The color change of the AgNo3 after its mixing with EH indicating the synthesis of AgNPs. It was observed that, the intensity of colour increased with increasing the incubation time and the concentration of $AgNO_3$. Absorption spectra of AgNPs formed in the reaction solution have shown absorbance peak at 425-450 nm at the concentrations, 20-50 mM of silver nitrate. **Fig (2 $_{b \& c}$)** have shown the absorbance values of AgNPs synthesized from 20 and 30 mM $AgNO_3$ which are 0.423 and 1.369, respectively. It is observed that the appearance of a new peak at 450 nm with absorbance value 1.264 in addition to the peak appeared at 425 nm with absorbance value 1.308 when 40 mM $AgNO_3$ has been used in the synthesis process (**Fig. 2$_c$**).

The UV-vis absorption corresponding to the reduction of the Ag^+ ions in aqueous solution of EH. There is no absorbance peak at the same wavelength which is detected in the solution of 5 and 10 mM after 3 days of incubation indicating the absence of nanoparticles at this concentrations.

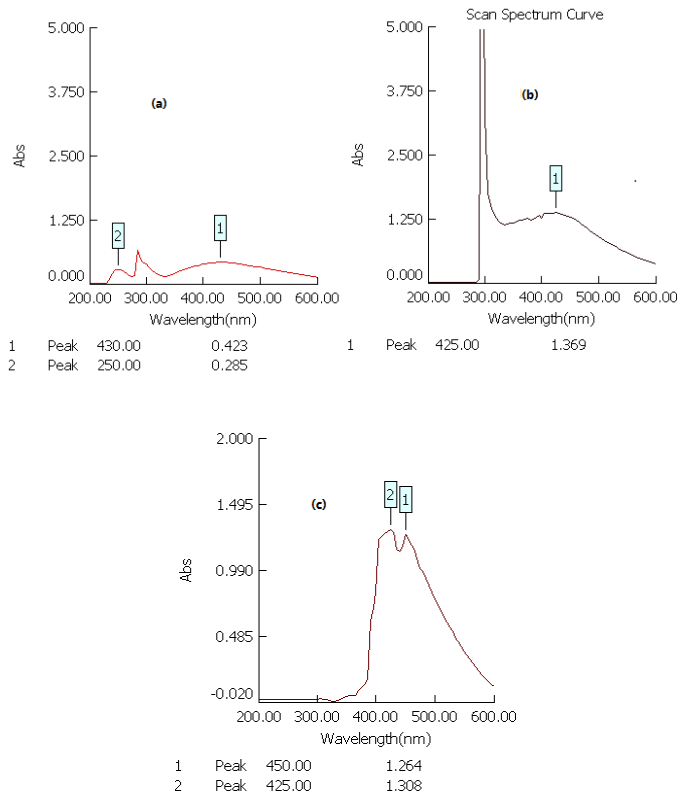

Fig 2: The UV/Vis spectrum of the silver nanoparticles synthesized by 10% EH at (a) 30 % (b) and 40% (c) of $AgNO_3$.

TEM and DLS studies

The TEM analysis has revealed that the size of AgNPs range between 9 and 22 nm **(Fig. 3)**. On the other hand, these results have been confirmed with size particles diffraction analysis (DLS) as shown in **(Fig.4)**. It has shown the average size distribution of AgNPs in colloidal solution which was found to be 9.99nm.

FTIR

The FT-IR spectrum (**Fig.5**) of honey AgNPs has revealed the evidence of nine bands at 3412.42, 2934.16, 1638.23, 1420.32, 1254.47, 1055.84, 917.95, 870.703, and 596.861.

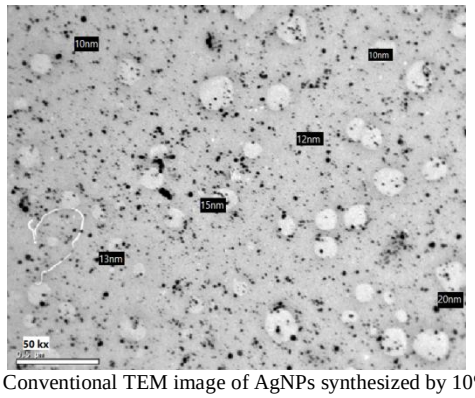

Fig 3: Conventional TEM image of AgNPs synthesized by 10% EH

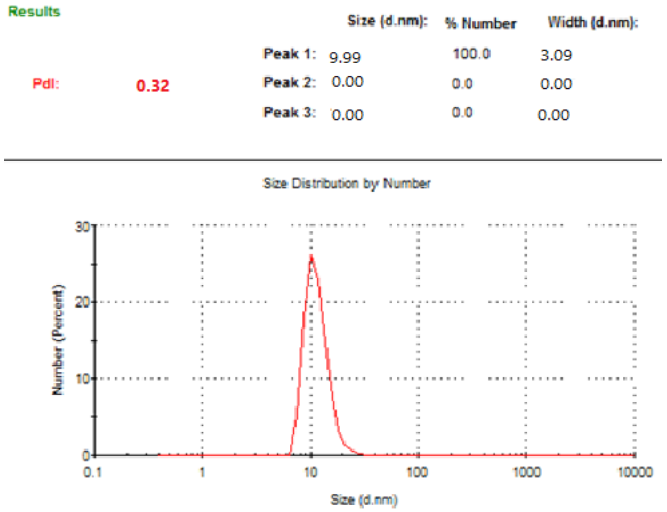

Fig 4: Dynamic light scattering measurements for particle size distribution analysis of AgNps prepared from 10% EH and 20 nm AgNO3.

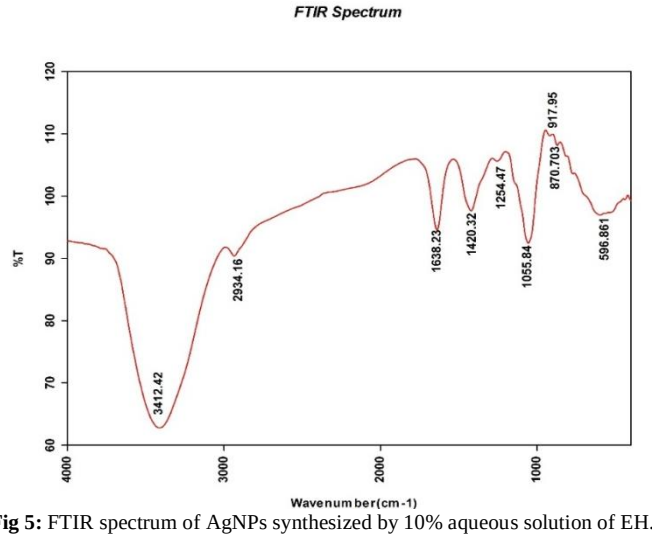

Fig 5: FTIR spectrum of AgNPs synthesized by 10% aqueous solution of EH.

Inhibitory effect of AgNPs against the growth of *Aspergillus* spp.

Data presented in Fig. (**6**) show the inhibition zone (mm) of fungal growth after treatment with EH- AgNPs colloids at the concentration 10, 20, 30 and 40 µg. The obtained data indicate that the highest level of inhibition was detected at the concentration 40 µg of AgNPs, where the inhibition zones are (24.2 ± 0.77) for *A. parasirticus* and (28.2 ± 1.04) for *A. ochraceous*. *A. parasiticus* shows higher inhibition level than *A. ochraceous* at all different concentration of AgNPs.

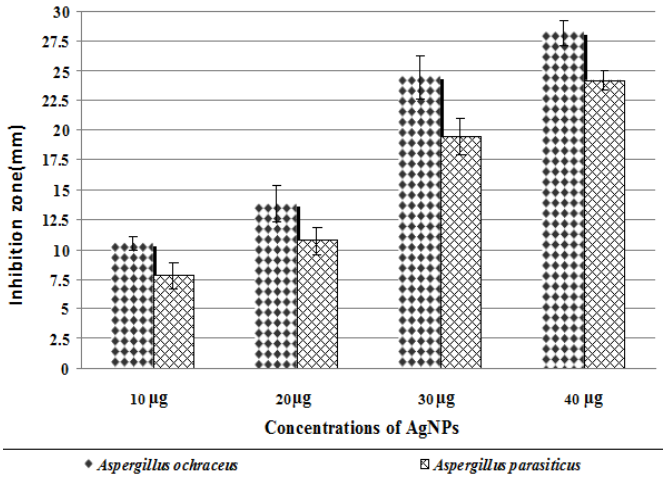

Fig 6: Effect of AgNPs synthesized by 10 % EH on mycelial growth of *Aspergillus ochraceus* and *Aspergillus parasiticus*. The bars on each column show standard deviation.

Evaluation of AgNPs synthesized by EH on production of AFs and OTA in YES medium

Data presented in (**Table** 1) show the concentrations of AFs in YES media treated with 1, 2and 3mgAgNPs$^{-100\ ml}$ media. In control media (without treatment) the concentrations of AFG_1, AFB_1, AFG_2 , AFB_2 and total AFs were 42.99±2.78, 53.76±1.32, 32.65±2.81, 27.31±1.7 and 156.73±5.6µg^{-100ml} media, respectively. While, in YES media treated by 1 mgAgNPs$^{-100\ ml}$ media the concentrations of AFs were reduced to 36.47±2.16, 43.63±2.7, 23.91±4.13, 18.11±2.04 and 122.12±4.32µg^{-100ml} media for AFG_1, AFB_1, AFG_2 , AFB_2 and total AFs, respectively. It is observed that the mycotoxins reduction increases significantly with the increment of AgNPs additives to YES media. Analysis of variance and Duncan analysis showed a significant ($p \leq 0.05$) between differences treatment on amount of AFs (Table 2).

The results displayed in **Fig (7**) show the percentages of reduction of AFs produced by *A. parasiticus* in YES medium, which was treated by different concentrations of AgNPs.

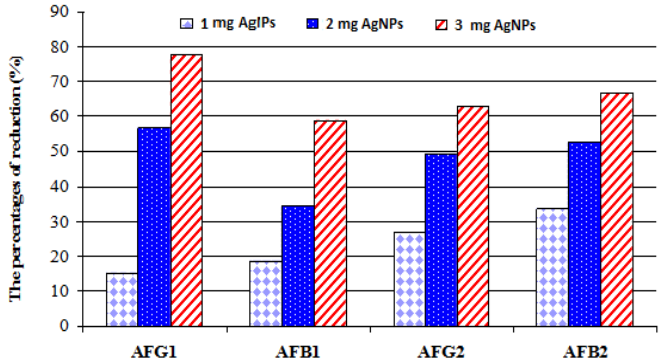

Fig 7: The percentages of reduction of AFs in liquid media treated with different concentration of AgNPs (1-3mg 100ml^{-1} culture medium).

The percentages of reduction of AFG_1, AFB_1, AFG_2 and AFB_2 in YES media treated by 3 mg AgNPs^{-100ml} media are 77.55, 58.76, 62.91 and 66.56%, respectively. On the other hand, the results indicate that the concentration of OTA produced by *A. ochraceus* was reduced to 7.93±1.26, 6.12±1.39 and 2.95±0.79in the culture media treated by 1, 2 and 3 mg $^{-100\ ml}$ culture medium, respectively as compared with control which was 14.64 ±0.71 µg^{-100ml} media **(Table 3)**.

The percentages of OTA reduction were 45.8, 58.2 and 79.85% after treating by 1, 2 and 3 mg AgNPs^{-100ml} media, respectively **(Fig.8)**.

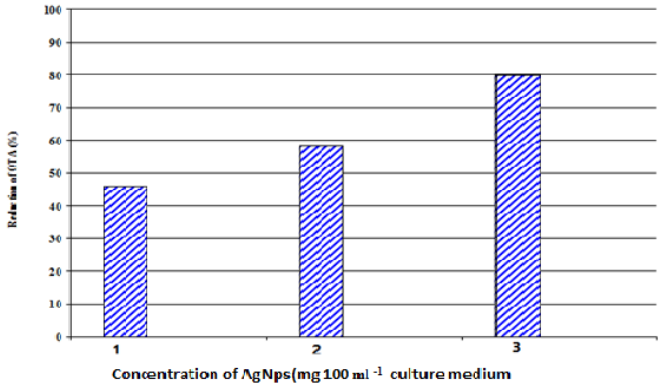

Fig 8: The percentages of reduction of OTA in YES media treated with different concentrations of AgNPs (1-3 mg 100 ml $^{-1}$ culture medium).

Table 1: Concentrations of AFs in liquid media treated by AgNPs colloids (Mean ± SD).

Concentrations of AgNPs mg $^{-100\ ml}$ media	Concentrations of AFs (µg $^{-100ml}$ media)*				
	AFG_1	AFB_1	AFG_2	AFB_2	Total AFs
Control	42.99±2.78^a	53.76±1.32^a	32.65±2.81^a	27.31±1.7^a	156.73±5.6^a
1	36.47±2.16^b	43.63±2.7^b	23.91±4.13^b	18.11±2.04^b	122.12±4.32^b
2	18.6±3.2^c	35.29±1.65^c	16.54±2.46^c	12.9±0.85^c	83.36±5.7^c
3	9.65±0.45^d	22.17±2.18^d	12.11±2.05^c	9.13±0.58^d	53.07±1.67^d

*Mean values in the column with the same letter are not significant different at 0.05 level.

Table 2: Analysis of variance of the effect of different concentration of AgNPs colloids on total AFs.

Source	SS	Df	MS	F	*P*
Intercept	129336.88	1	129336.88	5962.815	0.000
Concentration AgNPs	18386.655	3	6128.885	282.560	0.000
Error	173.525	8	21.691		
Total	147897.059	12			

SS: Sum of Squares, df: degree of freedom, MS: mean square, P: probability at confidence 0.95

Table 3: Concentrations of OTA in liquid media treated by AgNPs colloids (Mean ± SD)

Concentrations of AgNPs (mg^{-100ml} media)	Concentration of OTA (μg^{-100ml} media)*
Control	14.64 ± 0.71^{a}
1	7.93 ± 1.26^{b}
2	6.12 ± 1.39^{b}
3	2.95 ± 0.79^{c}

*Mean values in the column with the same letter are not significant different at 0.05 level.

DISCUSSION

Nanotechnology is one of the most promising and recent areas of research in modern science. The need of environmental synthetic protocols for the synthesis of nanoparticles leads to the developing interest on green chemistry approaches which are free from the use of toxic chemicals as by-products. Therefore, we have developed a simple, novel, green and economically feasible method for the synthesis of AgNPs using local EH and we have investigated their effect on growth and mycotoxin production by the mycotoxigenic strains, *A.parasiticus* and *A. ochraceus.* The first characterization of AgNPs has been by visual observation for color change of solution mixture. The brown color appeared in the reaction mixture of 10% EH containing (20, 30, 40 and 50 mM $AgNO_3$) after 72 h of incubation at 30 ^{0}C revealed the reduction of $AgNO_3$ into AgNPs. The intensity of color has increased with increasing the concentration of $AgNO_3$. This intensive colour of the reaction mixture at higher concentration of $AgNO_3$ indicates the formation of high amount of AgNPs.

The formation of AgNPs has been confirmed by UV-Visible spectroscopy which is one of the most widely used techniques for metal nanpparticles characterization (Philip 2010). Our results have shown the absorbance values of AgNPs synthesized from 20 and 30 mM $AgNO_3$ which are 0.423 and 1.369, respectively at the wave length 425-430 nm. The absorbance values of AgNPs synthesized from 40 mM$AgNO_3$ are 1.308 and 1.264 at the wave length 425 and 450 respectively. The appearance of these two peaks indicates the intensity of AgNPs formed at this concentration. This can reveal that the biomolecules presented in the EH is very strong to reduce the highest concentration of $AgNO_3$ (40 mM) into AgNPs without increasing concentration of Hoeny (10 %). These absorptions are attributed to the surface plasmon resonance (SPR) of the nanoparticles resulting from the reduction of the Ag^+ ions in aqueous solution of EH. The broadening of the peak at that wavelength is attributed to the formation of polydispersed silver nanoparticles in the solution mixture (Ponarulselvam *et al.,* 2012). The absorption peak, appeared at shorter wave length, is due to the presence several organic compounds of EH which are known to interact with Ag^+ ions. There is no absorbance peak appeared at the same wavelength in the solution of 5 and 10 mM AgNPs after 3 days of incubation indicating the absence of nanoparticles at this concentrations. Previous studies showed similar results for the synthesis of AgNps by using honey as a reducing and capping agent (Haiza *et al.,* 2013; Obota *et al.,* 2013).

The TEM analysis of synthesized AgNPs has detected the presence of well dispersed spherical nanoparticles over honey matrix with size range of 9-22 nm. No agglomeration of AgNPs has been observed from the TEM image indicating the presence of different biomolecules in the honey responsible for stabilizing the synthesized AgNPs. The size of AgNPshas been confirmed with size particles diffraction analysis (DLS) which has shown the average size distribution of AgNPs in colloidal solution to be 9.99 nm. Sreelakshmi *et al.,* (2011) agree with our results, who synthesized spherical AgNPs from Honey bee with size of11.9 nm (±5.25 nm). On the other hand, El-Deeb *et al.,* (2015) have synthesized spherical shape AgNPs from the bee insect.

FTIR analysis has been employed to detect the biomolecules presented in honey AgNPs. The broad bands appeared at 3412.42 corresponds the O–H stretch of phenols. The bands appeared at: 1638.23 and 870.703 are corresponding to N–H bend of amines, 1254.47 and 1055.84 to C–N stretch of aromatic and aliphatic amine respectively, 2934.16 and 917.95 to O–H stretch of carboxylic acids, 1420.32 to C–C stretch (in–ring) of aromatics, and 596.861 to C–Br stretch of alkyl halides. These functional groups indicate the complex composition of EH which may be responsible for the reduction process of AgNO3 into AgNPs. The Proteins presented in honey solution can bind to Ag nanoparticles through free amine groups or carboxylate ion of amino acid residue in it (Ponarulselvam *et al.,* 2012). Philip (2010) recorded that the possible reducing agent presented in honey is glucose and the capping material responsible for stabilization is proteins. Philip (2010) mentioned that honey contains at least 181 different substances including proteins, enzymes, amino acids, minerals, vitamins and polyphenols.

The antifungal activity of EH-AgNPs against *A. ochraceus* and *A. parasiticus* has been investigated. It has observed that the inhibition level of both strains increase with the increment AgNPs concentration at all different conditions. Similar results were investigated by Pulit *et al.,* (2013).They reported that

the higher AgNPs concentration induced stronger reduction of fungal growth. Although most of previous studies have evaluated the antibacterial activity of AgNPs, the antifungal activity of metal nanoparticles against filamentous fungi still limited. The antibacterial and antifungal activity of honey derived nanoparticles were previously demonstrated by Sreelakshmi *et al.,* (2011). The antifungal activity of AgNPs against *Cladosporium cladosporoides* and *Aspergillus niger* have been studied by Pulit *et al.,* (2013). They revealed that AgNPS at the concentration of 50 ppm inhibited the growth of *Cladosporium cladosporoides* and *Aspergillus niger* by 90% and 70%, respectively. Significant inhibition in mycelial growth of *Colletotrichum* supplied, with 100 ppm silver nanoparticles on PDA, was reported by Lamsal *et al.*, (2011). The inhibitory effect of AgNPs on fungal growth may be due to alteration of permeability of cell membrane, release of lipopolysaccharides and membrane proteins, generation of free radicals responsible for the damage of membrane, and dissipation of the proton motive force resulting in the collapse of the membrane potential (Kim *et al.*, 2007).

Likewise, our results show that AgNPs have significant effect on aflatoxin and OTA production by *A. parasiticus* and *A. ochraceus*, respectively. The concentration of AFG_1, AFB_1, AFG_2 and AFB_2 in YES media treated by 3 mg $AgNPs^{-100ml}$ media have been strongly decreased to 77.55, 58.76, 62.91 and 66.56%, respectively. Also, the concentration of OTA has been decreased to 45.8, 58.2 and 79.85% after the addition of 1, 2 and 3 mg $AgNPs^{-100ml}$ media, respectively. The decrement percentage depends on AgNPs concentration. The highest amount of AgNPs result in high reduction in aflatoxin and OTA production.

Similar studies on the effect of metal nanoparticles on the production of mycotoxins have been investigated. The inhibitory effect ZnO-NPs on the production of AFB_1 by *A. flavus* reported previously by Savi *et al.* (2013). There are no previous studies on the reduction effect of honey derived AgNPs on aflatoxin or OTA production by toxigenic fungi. Only few studies on the effect of biosynthesized AgNPs on mycotoxin production have been recorded **(**Yehia and Ahmed 2013, Al-Othman *et al.*, 2014).Al-Othman *et al.*, (2014) have investigated the effect of AgNPs biosynthesized by *Aspergillus terreus* (KC462061) on growth and aflatoxin production by *A. flavus* isolates. They found that The inhibition of AFB_1 production was 48.2 -61.8%, at 50 ppm , 46.1 - 82.2% at 100 ppm and 100% at 150 ppm AgNPs, while the inhibition of fungal growth was 100% at the concentration of 150 ppm AgNPs. That is the first investigation which evaluates the effect of AgNPs on OTA production by mycotoxigenic fungi.

Finally from this study, we can conclude that the AgNPs with small size can be synthesized by nontoxic ecofriendly method using 10% EH and their formation depend on the concentration of AgNPs. We have also shown, for the first time, the inhibitory effect of honey derived AgNPs on fungal growth of *A. parasiticus* and *A.ochraceus* and its strong inhibitory effects on AFs and OTA production. The highest concentration of AgNPs gives the highest percentage reduction of AFs and OTA. This investigation required extensive researches to can be applied on the detoxification of AFs and OTA in food and feed stuff.

Conflict of interest

The authors declare that there are no conflicts of interest.

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