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Green synthesis of silver nanoparticles using *Morus nigra* leave extract and evaluation their antifungal potency on phytopathogenic fungi

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ABSTRACT

This study aimed to isolate and identify the fungi associated with white and yellow corn grain and peanuts seeds samples collected from different distracts at Cairo, to use *Morus nigra* leaves extract for the green synthesis of silver nanoparticles (AgNPs) and to evaluate the antifungal potency and anti-aflatoxin production of the synthesized AgNPs. White and yellow corn and peanut seeds samples were collected from 4 different districts of Cairo, mycoflora were isolated on Potato dextrose agar (PDA), Nash and Coon's media. The results revealed the isolation of sixteen fungal species belonging to 7 genera were recovered and identified during this study. The produced AgNPs showed Uv/Vis absorbance at 425 nm and particles sizes ranged from 4 to 8 nm. Both AgNPs and the plant extract showed a strong antifungal activity against *P. carryophylum*; *F. verticillioides*; *A. flavus; A. terreus* and *F. oxysporum* using different techniques. Moreover, AgNPs was more effective than the plant extract against the tested fungi and anti-aflatoxin production by *A. parasiticus*. It could be concluded that the *Morus nigra* leaves extract can be used for the syntheses of AgNPs and also as antifungal of plant disease. Thus, *Morus nigra* and AgNPs could be used as effective, safe and ecofriendly antifungals to prevent fungal growth and subsequent aflatoxins production.

INTRODUCTION

Seed-borne fungi have been found to affect the growth and productivity of crop plants including legumes and cereals (Weber *et al*., 2001), in addition some species can produce health-damaging mycotoxins. The mycotoxins contamination in animal feed and human food represent a serious health and economic problem worldwide. Mycotoxins are secondary metabolites of several species of fungi. They are unavoidable and their production is depending on the environmental factors either in the field or during storage (Stoev, 2013). The majority of mycotoxins are potentially carcinogenic, tremorogenic, teratogenic, immunotoxic, nephrotoxic or hemorrhagic beside most of them cause dermatitis in both livestock and humans (Bhat and Reddy, 2017).

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Among all mycotoxins, aflatoxin B_1 (AFB_1) is known to be the most significant in terms of animal and human health risk (CAST, 2003; Bryden, 2012). The control of phytopathogenic fungi is the first step towards the protection of cereal crop programs. Though successful, however, synthetic fungicides have main disadvantages such as; the accumulation in the ecosystem and the development of pesticide resistance pathogens (Okigbo, 2004).

Plants produce enormous array of secondary metabolites which serves to protect them against different pathogens. Mulberry (*Morus nigra*, Family: Moraceae) leaves have been used for the treatment of inflammation, hyperglycemia, cough, and fever (Radojković *et al*., 2016) due to its high content of phenolics with strong antioxidant activities which are thought to contribute to the biological activities of these leaves (Naowaratwattana *et al*., 2010). A problem with the use of plant-produced compounds as potential fungicides, is that in their natural state they are generally weakly active compared to synthetic fungicides (Gisi, 2014).

Recently, metal nanoparticles have acquired a great attention because of their noteworthy properties, which are largely different than the original metals (Li *et al*., 2015; Xu *et al*., 2013).

Silver nanoparticles (AgNPs) have a great recognition due to several superior properties and their widely application in various fields (Yan *et al*., 2014; Nemanashi and Meijboom, 2013). However, the applications are dependent mainly on their size, shape, sizes distribution and stability of AgNPs in aqueous solution (Abdel-Aziz *et al*., 2014). Several strategies chemical and physical methods were developed for the synthesis of AgNPs included chemical reduction, radiation, microwave-assisted synthesis, ultrasonic irradiation, photo reduction, electrochemical reduction and chemical reduction (Dong *et al*., 2016; 2014, Rabinal *et al.,* 2013).

However, the chemical reduction of silver ions method to metallic silver in the presence of capping agents was reported to be the most popular method for the synthesis of AgNPs (Biçer and Şişman, 2010). Most of the capping agents or reducing agents used in these chemical methods may induce toxic effect resulting in risks to the environment (Oluwafemi *et al*., 2013). Moreover, the use of such environmental toxic chemicals may limit the application of AgNPs. Consequently, to minimize these environmental hazardous chemicals and to maximize the safety and efficiency of AgNPs, several approaches were developed to prepare AgNPs using green chemistry (Oluwafemi *et al*., 2013).

The plant extract is one of the most promising method used as stabilizing and reducing agents. The leaf extract of several plants were used for the synthesis of AGNPs including *Gloriosa superba* L. (Ashokkumar *et al*., 2013), *Prosopis juliflora* (Raja *et al*., 2012), Rosadamascene (Ghoreishi *et al*., 2011, *Sesbania grandiflora* (Das *et al*., 2013), *Rosa rugosa* (Dubey *et al*., 2010), *Hibiscus cannabinus* (Bindhu *et al.,* 2013), *Barbated Skullcup* herb (Wang *et al*., 2009), *Anacardium occidentale* (Philip and Unni, 2011).

The objectives of the current study were to use mulberry aqueous leaf extract for the green synthesis of AgNPs and to evaluate the antifungal activity and anti-aflatoxin production of both, the plant extract and the synthesized AgNPs.

MATERIALS AND METHODS

Plant materials

Ten samples of stored white and yellow grains and fifteen groundnut pods (400 g each) were collected from 4 different districts at Cairo governorate, Egypt, namely; Gesr El-Suez; Hadeik El-Kobba; El-Zeitoun and El- Haram. Mulberry (*Morus nigra*, Family: Moraceae) leaves were collected from agricultural areas in Dekernis districts, Mansoura, Egypt during June 2013.

Preparation of plant extract and synthesis of AgNPs

Twenty-five gram of fresh clean leaves of mulberry were used for preparation of aqueous extract according to Abdel-Aziz *et al*., (2014). Silver nanoparticles (AgNPs) were synthesized using the mulberry leaf extract as described by Parashar *et al*., (2009).

Characterization of AgNPs

UV-vis adsorbance spectroscopy analysis

The bioreduction of silver nitrate ($AgNO_3$) to AgNPs was monitored periodically by UV-vis spectroscopy (Shimazu 2401PC, Mundelein, Il, USA) after the dilution of the samples with deionized water (Raut *et al*., 2009). The UV-vis spectrometric reading was recorded at a scanning speed of 200 to 800 nm with water (Leela and Vivekanandan, 2008).

TEM analysis of silver nanoparticles

The suspension containing AgNPs was sampled by TEM analysis using JEOL model 1200 EX electron microscope (München, Germany). The shape and size of AgNPs were determined from TEM micrographs in reference to Elavazhagan and Arunachalam, (2011).

Isolation and identification of seed borne mycoflora

Isolation of the seed-borne mycoflora associated with internal seeds of groundnut, yellow and white corn grains was carried out according to the standard agar plate method described by the International Seed Testing Association (ISTA, 2003), on Potato dextrose agar (PDA), Nash and Coon's media. Fungi were macroscopically and microscopically identified on the basis of their typical structure and basic characters as suggested by Melone and Masket, (1964); Barnett and Hunter (1998). Aflatoxigenic isolate of *Aspergillus parasiticus* was provided by Plant Pathology Department, National Research Center, Giza, Egypt.

In vitro evaluation of the antifungal potency and MIC of mulberry leaf extract and AgNPs against the selected mycoflora

Aqueous mulberry leaf extract with different concentrations (0.1, 0.2, 0.4 and 0.8%) was tested for *in vitro* antifungal potential against the selected fungal pathogens by poisoned food technique and agar well diffusion assay following the modified procedure of Mohana and Raveesha, (2010); Khyade and Vaikos, (2009), respectively. *In vitro* assay of the antifungal activity of AgNPs was performed on PDA growth medium treated with different concentrations (i.e., 0.1, 0.2, 0.4, 0.8% and 1.6%) of AgNPs using the same above mentioned assays according to Kim *et al*., (2012); Devi and Bhimba, (2014), respectively. Dithane M-45 was used as a positive control antifungal and three replicates of each treatment were performed.

Minimum inhibitory concentrations (MIC) of the extract was determined using poisoned food technique according to Yanar *et al*., (2011) using various concentrations of the extract (0.1, 0.2 and 0.4 %), whereas, MIC of the AgNPs was conducted as described by Hassan *et al*., (2007) using agar well diffusion method using 0.1 and 0.2% concentrations only.

Effect of mulberry leaf extract and AgNPs on mycelial dry weight of selected mycoflora

The reduction of dry weight of fungal mycelia of the selected mycoflora due to activities of the mulberry extract and AgNPS were conducted as described by Venturini *et al*., (2002); Agrawal *et al*., (2004), respectively, using different concentrations mainly 0.1, 0.2, 0.4, 0.8 and 1.6%.

***In vivo* estimation of the anti-aflatoxin production activities of the leaf extract and AgNPs**

The *in vivo* anti-aflatoxin potency of the plant extract and AgNPs on aflatoxin B_1 (AFB_1) production in corn grains infested with the aflatoxigenic *A. parsiticus* was determined following the procedures of Garcia *et al*., (2012). However, AFB_1 extraction and quantification were conducted according to Singh *et al*., (1991); VICAM (1999); Shukla *et al*., (2008) using HPLC analysis.

Statistical analysis

All data were statistically analyzed using the General Linear Model Procedure of the Statistical Analysis System (SAS 1982). The significance of the differences among treatment groups was determined by Waller–Duncan k-ratio (Waller and Duncan, 1969). All statements of significance were based on probability of $P \leq 0.05$.

RESULTS

Isolation of mycoflora from stored maize grains and groundnut seeds

The results of isolation and identification of seed borne mycoflora revealed that 16 different fungal species belonging to 7 genera were obtained mainly: *Fusarium verticillioides*; *F. oxysporum*; *Fusarium* spp.; *Aspergillus flavus*; *A. niger*; *A. terreus*; *A. parasiticus*; *A. candidus*; *A. tamari*; *A. sydowii*; *Penicillium carryophylum*; *Alternaria* sp.; *Mucor* spp.; *Gliocladium fimbriatum*; *Rhizopus stolonifer* and *Rhizopus* sp. Five isolates known of being pathogens and mycotoxins producers mainly; *A. flavus; A. terreus; F. oxysporum; F. verticillioides* and *P. carryophylum* were selected for further research, whereas, the remaining isolates were neglected.

Results in Table 1 showed that the number of fungal count isolated from white corn samples collected from Gesr El-Suez district using PDA medium was the highest (409 CFU/ 100 seeds) with infection ratio of 90.90 % followed by the samples collected from Hadeik El-Koba which recorded 250 CFU/ 100 seeds with infection ratio of 68.75 %.

However, the samples collected from El-Haram recorded TFC of 75 CFU/ 100 seeds with infection ratio of 18.75 % and the samples collected from El-Zeiton recorded 50 CFU/ 100 seeds with infection ratio of 12.5 %. The yellow corn samples collected from Hadik El-Koba was found to be the most fungal infected samples and the recorded TFC using PDA medium was 450 CFU/ 100 seeds followed by the samples collected from Gesr El-Suez which recorded 233 CFU/ 100 seeds, then those collected from El Zeiton and El-Haram which recorded 150 CFU/ 100 and 100 CFU/ 100 seeds, respectively. The percentage of infection for the 4 districts were 100, 75, 31.25 and 6.25 % for the samples collected from Hadik El-Koba, Gesr El-Suez, El-Zeiton and El-Haram, respectively.

Moreover, the recorded TFC in groundnut samples collected from the four districts using PDA were 225, 450, 118 and 113 for Gesr El-Suez, Hadeik El-Koba, El-Zeiton and El-Haram, respectively with percentages of infection reached 81.25, 100, 25 and 50 % respectively.

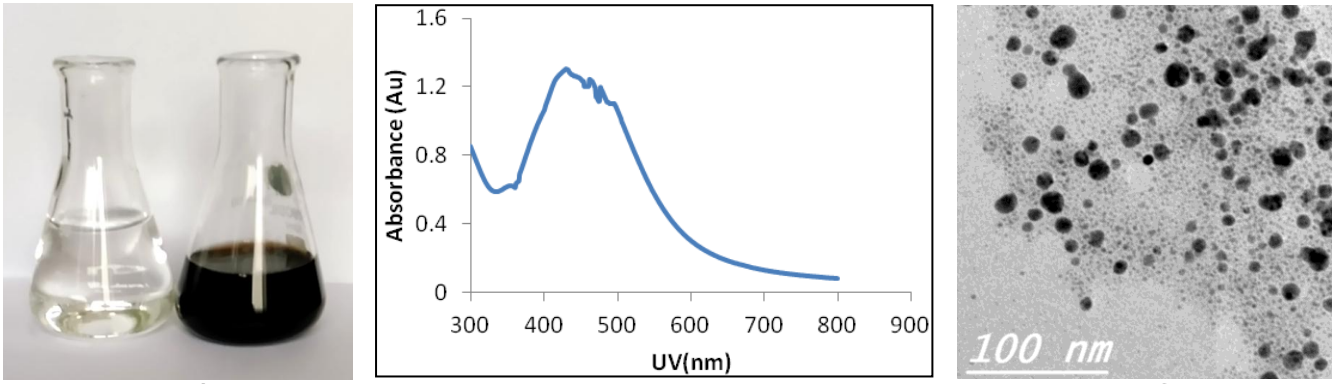
The results presented in Table (1) also revealed that TFC isolated from white corn using Nash medium recorded 362, 106, 69 and 32 CFU/ 100 seeds for Gesr El-Suez, Hadeik El-Koba, El-Zeiton and El-Harm, respectively which were corresponding to 93.75, 43.75, 18.75 and 12.5 % for these districts, respectively. Using the same medium, TFC in yellow corn samples collected from the 4 districts recorded 208, 325, 57 and 250 CFU/ 100 seeds with percentages of infection reached 66.66, 75, 12.5 and 62.5% for the samples collected from Gesr El-Suez, Hadeik El-Koba, El-Zeiton and El-Haram, respectively.

TFC recorded for groundnut samples collected from the four districts using Nash medium were 275, 408 and 25 for the samples collected from Hadik El-Koba, Gesr El-Suez and El-Haram, respectively, however; the groundnut samples collected from El-Zeiton did not record any infection using this medium. However, the TFC recorded for white corn using Coon´s medium isolated from the samples collected from Gesr El-Suez, Hadeik El-Koba, El-Zeiton and El-Haram were 368, 62, 75 and 125, respectively with recorded percentages of infection reached 93.75, 37.75, 18.75 and 18.75% for the four districts, respectively.

On the other hand, the TFC recorded in yellow corn collected from the 4 districts using Coon´s medium were 377, 441, 144 and 125 CFU/ 100 seeds for the samples collected from the 4 studied areas, respectively. The recorded infection percentages were 93.75, 75, 37.5 and 43.75 for the same districts, respectively. The recorded TFC for groundnuts samples collected from these districts using Coon´s medium were 266, 400, 168 and 663 CFU/ 100 seeds with infection ratio reached 100, 93.75, 37.5 and 100 %, respectively.

Characterization of AgNPs

Addition of mulberry leaf extract to silver nitrate (1mM) in darkness resulted in changing its color to brownish indicating the formation of AgNPs, (Fig. 2a) and the produced solution showed Uv/Vis absorbance at 425 nm (Fig. 1b). Transmittance electron microscope (TEM) studies approved that the diameter of the nanoparticles ranged from 4 to 8 nm (Fig. 1c).



A B C

Fig. 1: (a) Color development of silver nitrate to AgNPs (brown) by the addition of mulberry leaf extract; (b) The spectrum of AgNPs synthesized by aqueous leaf extract of mulberry detected through Uv/Vis absorbance and (c) TEM photograph of AgNPs biosynthesized by mulberry leaf extract.

Table 1: Percentage of fungal infection and number of total fungal counts (CFU/100 seeds) associated with white, yellow corn grains and groundnut seeds collected from 4 locations in Cairo on three different media.

Location	Media	White corn		Yellow corn		Groundnut	
		Infection %	CFU/100	Infection %	CFU/100	Infection %	CFU/100
Gesr El-Suez	PDA	90.90	409	75	233	81.25	225
	Nash	93.75	362	66.66	208	87.5	275
	Coon´s	93.75	368	93.75	337	100	266
Hadeik El-Koba	PDA	68.75	250	100	450	100	450
	Nash	43.75	106	75	325	83.33	408
	Coon´s	37.5	62	75	441	93.75	400
El-Zeiton	PDA	12.5	50	31.25	150	25	118
	Nash	18.75	69	12.5	57	0	0
	Coon´s	18.75	75	37.5	144	37.5	168
El-Haram	PDA	18.75	75	6.25	100	50	113
	Nash	12.5	32	62.5	250	25	63
	Coon´s	18.75	125	43.75	125	100	663

Table 2: Mean diameter of inhibition of radial growth of the tested fungi treated with the plant extract and AgNPs using poisoned food technique.

Fungi	Control	Fungicide		Plant extract				AgNPs			
		0.8 %	1.6 %	0.1 %	0.2 %	0.4 %	0.8 %	0.1 %	0.2 %	0.4 %	0.8 %
A. flavus	84.33 ±0.47	0	0	81.6 ±0.24	79.6 ±0.24	49.6 ±2.01	26.3 ±0.63	74.3 ±0.63	31.3 ±0.47	29.3 ±0.24	20.6 ±0.24
A. terreus	80.0 ±0	0	0	74.0 ±0.82	53.6 ±0.85	40 ±0.41	22.0 ±0.41	74.0 ±0.82	53.6 ±0.85	40.0 ±0.41	22.0 ±0.41
F. oxysporum	90.0 ±0	0	0	64 ±1.081	55.0 ±1.081	40.0 ±0.41	28.0 ±0.41	54.3 ±0.85	42.3 ±0.63	32.0 ±0.41	21.3 ±0.24
F. verticillioides	90 ±0	0	0	64 ±1.081	61.6 ±0.623	55.3 ± 1.93	55.3 ± 0.849	60.0 ±0	57.3 ±0.235	54.5 ±0.250	49.0 ±0.41
P. carryophylum	80.66 ±0.63	0	0	61.66 ±0.24	31.0 ±0.41	26.33 ±0.63	23.33 ±0.63	41.33 ±0.63	23.33 ± 0.24	22.0 ±0.41	20.33 ±0.24

Values are averages of three replicates for each treatment ± SE.

Table 3: Mean diameter of inhibition of radial growth of the tested fungi treated with the plant extract and AgNPs using Agar well diffusion.

Fungi	Control	Fungicide	Plant extract					AgNPs				
		1.6 %	0.1 %	0.2 %	0.4 %	0.8 %	1.6 %	0.1 %	0.2 %	0.4 %	0.8 %	1.6 %
A. flavus	0	36.0 ± 0.41	0	0	0	5.66 ± 0.24	9.33 ± 0.24	0	0	6.66 ±0.24	8.33 ± 0.24	11.6 ± 0.63
A. terreus	0	33.66 ± 0.63	0	0	0	10.0 ± 0.41	7.33 ± 0.24	0	0	11.33 ± 0.63	11.0 ± 0.41	7.66 ± 0.24
F. oxysporum	0	41.66 ±0.47	0	0	8.0 ±1.18	13± 0.41	20.3± 0.63	0	9.3 ± 0.47	7.6 ± 0.24	24.0 ± 0.41	30.3 ± 0.47
F. verticillioides	0	42.33±0.63	0	0	7.0 ± 0.41	16.3± 0.85	20.6 ± 0.24	0	7.6 ± 0.24	16.0 ± 0.41	20.6 ± 0.63	25.6 ± 0.85
P. carryophylum	0	41.67 ± 0.47	0	0	5.67 ± 0.24	6.33 ± 0.24	9.0 ±0.41	0	8.0 ± 0.41	11.0 ± 0.41	12.75± 0.48	18.0 ± 0.82

Values are averages of three replicates for each treatment ± SE.

In vitro antifungal potential of the mulberry extract and AgNPs on the selected mycoflora

The current results demonstrated that the antifungal potency of the plant extract and AgNPs against the selected fungi was increased with increasing their corresponding concentrations. In poisoned food technique (Table 2), the mean diameter of radial growth of all tested fungi was decreased significantly in a dose dependent manner after the addition of the plant extract or AgNPs.

However, synthetic fungicide caused complete inhibition of the growth of all fungal species at concentration of 0.8% and 16%. Moreover, these results showed that AgNPs was more effective than the plant extract. In agar well diffusion assay (Table 3), the inhibitory activities of the AgNPs against the radial growth of *P. Carryophylum* was almost double that of the plant extract at all tested concentrations. However, AgNPs could inhibit the radial growth of the other tested fungi in a dose dependent fashion.

Determination of the minimum inhibitory concentrations (MIC) of plant extract and AgNPs against seed borne fungi

The minimum inhibitory concentrations (MIC) of the plant extract and AgNPs causing MIC are shown in Table (4). In poisoned food technique, the MIC of plant extract and AgNPs against the five selected seed borne fungi was 0.1%. This low concentration demonstrates the high antifungal activities of plant extract and the AgNPs.

Table 4: Minimum inhibitory concentrations (MIC) of plant extract and AgNPs against seed borne fungi using poisoned food and agar well diffusion techniques.

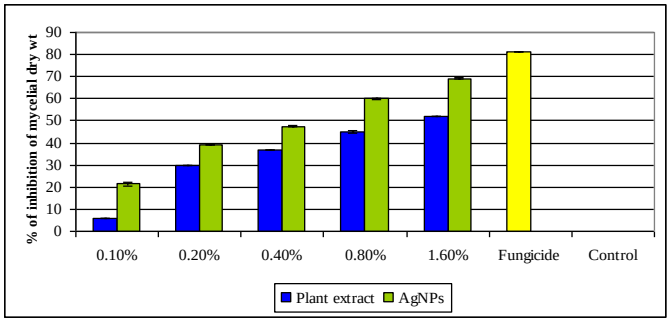
Seed borne mycoflora	Minimum Inhibitory Conc. (MIC)			
	Poisoned food technique		Agar well diffusion technique	
	Plant extract	AgNPs	Plant Extract	AgNPs
A. flavus	0.10%	0.10%	0.80%	0.40%
A. terreus	0.10%	0.10%	0.80%	0.40%
F. verticillioides	0.10%	0.10%	0.40%	0.20%
F. oxysporum	0.10%	0.10%	0.40%	0.20%
P. carryophylum	0.10%	0.10%	0.40%	0.20%

In case of agar well diffusion technique, the MIC of the plant extract against *F. verticillioides*, *F. oxysporum* and *P. carryophylum* was 0.4%, whereas, it was 0.8% against *A. flavus*, *A. terreus* which revealed that the high concentrations demonstrating the low activity of the plant extract in this used technique. On the other hand, the MIC of AgNPs was 0.2% against *F. verticillioides*, *F. oxysporum* and *P. carryophylum*, and 0.4% against *A. flavus*, *A. terreus*.

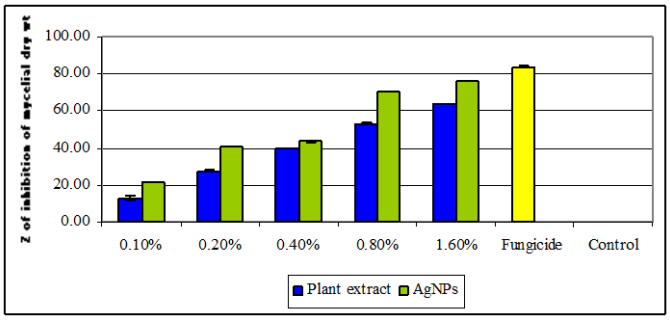
These results demonstrated the higher activity of AgNPs against seed borne fungi compared with the plant extract, higher resistance of *A. flavus* and *A. terreus* to both agents, on the contrary to *F. verticillioides*, *F. oxysporum* and *P. carryophylum* which showed more sensitivity. In addition, these results demonstrated also that the poisoned food technique was more sensitive and accurate than the agar well diffusion technique.

Effect of the mulberry extract and AgNPs on mycelial dry weight of the selected mycoflora

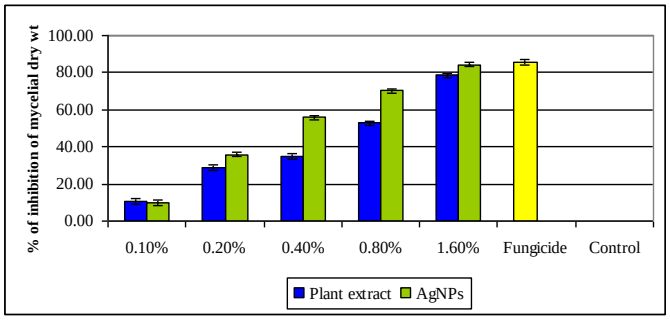
The inhibitory effects of the plant extract and AgNPs on the mycelial dry weight of the tested fungi (Fig. 2) revealed that the inhibition of mycelia dry weight was increased with increasing their corresponding concentrations. The synthetic fungicide caused higher inhibition percentage (82.20%), compared with the same concentration of both agents. This inhibition of myceial dry weigh was more pronounced with AgNPs for *A. flavus*, *A. terreus* and *P. carryophylum* at all tested concentrations tested. However, the plant extract at 0.1% showed an insignificant increase in mycelia dry weight for *F. verticillioides* and *F. oxysporum*.



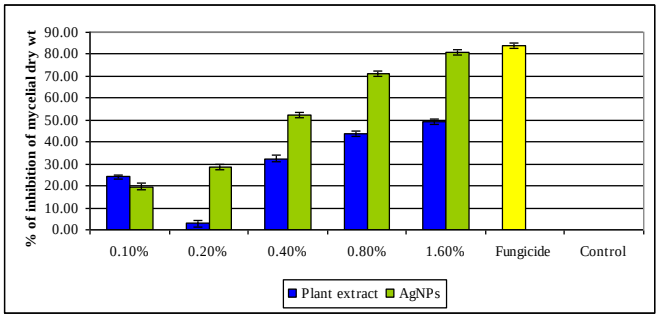
A. flavus



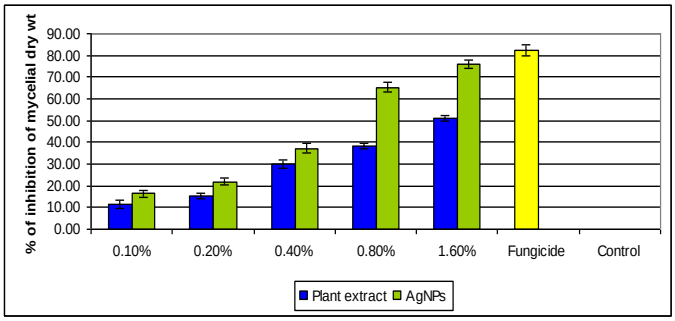
A. terreus



F. verticillioides



F. oxysporum



P. carryophylum

Fig. 2: Effect of plant extract and AgNPs on mycelial dry wt. of the tested fungi.

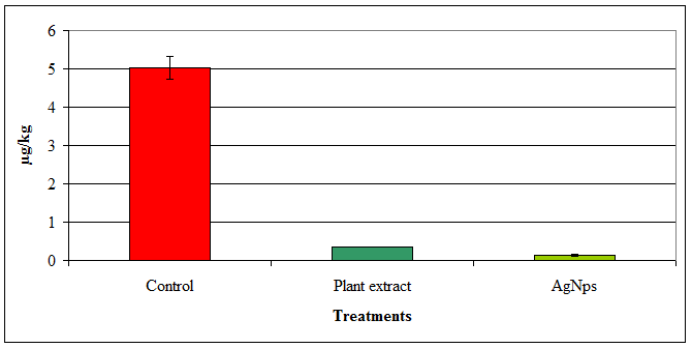


Fig. 3: Total aflatoxin concentration in non-treated corn grains and grains treated with the plant extract or AgNPs.

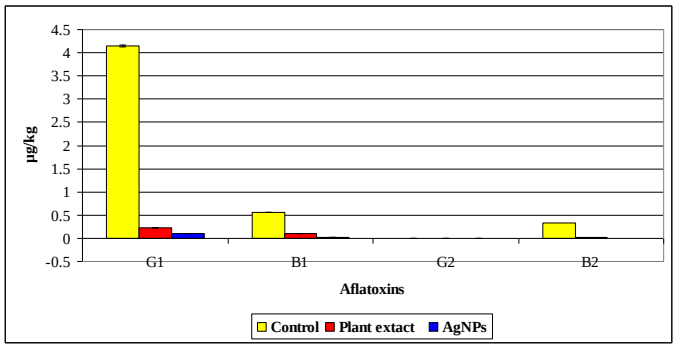


Fig. 4: Concentration of aflatoxins in corn grains infested with *A. parsiticus* only and those treated with the plant extract or AgNPs

Anti-aflatoxin production in corn grains treated with the plant extract and AgNPs

The HPLC analysis of aflatoxin in the infested corn grains revealed the presence of total aflatoxins in a concentration reached 5.03 µg/ kg corn grain sample, however, the concentration of total aflatoxin in the infested corn grain treated with the plant extract or AgNPs reached 0.35 and 0.13 µg/ kg, respectively (Fig. 3). Moreover; the data revealed the absence of AFG_2 in all treated and untreated samples, whereas, AFB_2 was only absent in the samples treated with AgNPs (Fig. 4).

DISCUSSION

In the current study, the isolation and identification of seed borne mycoflora showed that 16 different fungal species belonging to 7 genera were obtained mainly: *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria*, *Mucor*, *Gliocladium* and *Rhizopus*. The results also revealed that all the yellow and white corn grains and peanuts seeds collected from different districts in Cairo are infected with these fungal species which may be due to the bad storage conditions. Similar to the current results, Madbouly *et al*., (2012) reported that 23 species belonging to 12 different genera of fungi were isolated from corn grains collected from different districts of Cairo governorate, the percentages of infection of corn ranged from 16% to 142%. About 70% of these samples were infected with *Aspergillus flavus* and *Aspergillus niger* with percentages of 33%, 40%, respectively.

Biological synthesis of AgNPs using plant extracts is safe and has no phytotoxic effects (Gardea-Torresdey *et al*., 2003). Aqueous silver ions were reduced to AgNPs after mixing with mulberry leaves and change in color to reddish brown has been previously observed (Khandelwal *et al*., 2010) which was suggested to be due to the surface plasmon resonance of deposited AgNPs. Following the TEM study, the size of the nanoparticles ranged approximately from 4 to 8 nm, which are in the size range to impart higher antimicrobial effect (Khadri *et al*., 2013).

The antifungal potency of the plant extract and AgNPs were examined against the five selected seed-borne fungi, using poisoned food and agar well diffusion techniques.

In all treatments, AgNPs exhibited higher antifungal activity even at low concentration (0.1%), this could be attributed to the stability of the synthesized AgNPs. The antifungal potency of the plant extract and AgNPs increased with increasing their corresponding concentrations. However the sensitivity to this extract varied with different isolates which may be due to the difference in intrinsic tolerance of these isolates, in addition to the nature and combinations of phytocompounds present in this crude extract (Senguttuvan *et al.*, 2013). In accordance, Radojković *et al*., (2012) stated that mulberry root extract had high total phenolics contents (186.30 mg CAE/g), whereas, high total flavonoids content (67.37 mg RE/g) were determined for its leaf extracts. Whether these phenolic principles acted singly or synergistically, they might prevent and/ or decrease the growth of these fungi by disturbing their respiratory chains, denaturating enzymes and proteins within the fungal cells and might inhibit the biosynthetic pathways of mycotoxins in these fungi (Madbouly and El Magly, 2015). In poisoned food technique, *P. carryophylum* was highly sensitive to AgNPs and mulberry extract followed by *A. flavus*, conversely; *F. verticilliodes* was resistant to both agents as it recorded the highest diameter of radial growth. In agar well diffusion assay, *A. flavus* and *A. terreus* were sensitive only to high concentrations of the plant extract; however, they were resistant to its lower concentrations. The same with *Aspergilli* were also resistant to low concentrations of the AgNPs. On the other hand, at high concentration of both agents, *A. flavus*, *A. terreus* and *P. carryophylum* showed the least diameters of inhibition zones, indicating the higher resistance of these fungi to both control agents. Similar results were observed with *A. flavus; A. terreus; F. oxysporum* and *F. verticillioides*. On using the poisoned food technique, the MIC of the plant extract and AgNPs was very low about 0.1%, indicating the effectiveness of both agents against all selected seed-borne fungi. Meanwhile, in case of the agar well diffusion assay, the MIC of AgNPs against *F. verticilliodes*, *F. oxysporum* and *P. carryophylum* was 0.2%, however, it was 0.4% against *A. flavus* and *A. terreus*, therefore low concentrations of silver nanoparticles could inhibit growth of these mycoflora. Both agents caused considerable inhibition in the mycelial dry weight. of all selected fungi. *F. verticilliodes* showed

maximum sensitivity to the plant extract and AgNPs. Conversely, *F. oxysporum* showed maximum resistance to the plant extract (49.1%), whereas, *A. flavus* recorded high resistance to the AgNPs. These activities of both agents could be attributed to the denaturation of proteins and consequently inhibiting the action of enzyme systems responsible for growth of these fungi. This recorded high antifungal potency of the AgNPs might be because upon treatment with Ag^{+}, the DNA loses its ability to replicate (Feng *et al*., 2000), Leading to inactivated expression of ribosomal subunit proteins, as well as certain other cellular proteins and enzymes essential to ATP production (Yamanaka *et al*., 2005), hence inhibiting the biosynthetic pathways of aflatoxins in *A. parasiticus* isolate. Generally, nanopesticides represent the next-generation to traditional pesticides, as they have higher efficacy, durability and less doses of active ingredients (Khot *et al*., 2012). For the best of our knowledge, the current study is the first report of using mulberry leaf extract for the green synthesis of AgNPs.

Aflatoxins (AFs) are secondary metabolites with toxic and carcinogenic effects, produced by species of *Aspergillus*, particularly *A. flavus* and *A. parasiticus* (Razzaghi-Abyaneh *et al*., 2008). In the current study, the HPLC analysis of total aflatoxins present in corn grains co-inoculated separately with the plant extract, AgNPs and the aflatoxigenic isolate of *A. parasiticus* showed reduction of its level to 0.35 and 0.13 µg/ kg, respectively, compared with 5.03 µg/ kg in corn grains infested with the aflatoxigenic isolate only.

CONCLUSION

It could be concluded from the results of the current study that AgNPs could be green synthesized by mulberry leaf extract and then used effectively as safe, ecofriendly nano-fungicides to inhibit the fungal growth and subsequent aflatoxins production in cereal grains during storage.

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