



Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 7(12) pp. 377-382, December, 2018 Special Anniversary Review Issue.
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Full Length Research Paper

Antifungal activity of silver@silica nanoparticles against aflatoxigenic *Aspergillus flavus*

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Accepted 3 December, 2018

In Senegal, *Aspergillus flavus*, which develops on crops by producing aflatoxins, seriously affects the economy and the health of consumers. In this study, the antifungal effect of silver@silica on aflatoxigenic *A. flavus* was investigated. An aflatoxigenic *A. flavus* strain was isolated on Potato Dextrose Agar (PDA) medium from infested groundnut seeds. silver@silica nanoparticles were synthesized by reverse microemulsion and different concentrations of them were tested on *A. flavus*. The bioactivity type of silver@silica nanoparticles was also determined. The obtained results revealed that strong inhibition of *A. flavus* was noticed at all concentrations. Additionally, the bioactivity tests performed showed that silver@silica nanoparticles had a fungicide effect on *A. flavus*. Thus, silver@silica nanoparticles could be used as effective, safe and ecofriendly antifungals to prevent fungal growth and subsequent aflatoxins production.

Keywords: Aflatoxigenic *Aspergillus flavus*, antifungal activity, silver@silica nanoparticles, reverse microemulsion

INTRODUCTION

Fungi that develop on crops producing mycotoxins seriously affect the agri-food sector in sub-tropical countries. Their presence on crops has harmful effects on human and animal health. In addition, economic losses due to reduced market value and impaired seed nutritional and germinative qualities, as well as export restrictions to many countries must be considered (PACA, 2012). This

alarming fact concerns particularly the strains of fungi *Aspergillus parasiticus*, *nomius* and *flavus*, very prominent in legumes, grains and oilseeds such as cowpea, maize, sorghum and groundnut. These fungi secrete mycotoxins called aflatoxins. Among these mycotoxins, aflatoxin B1 is the most toxic and has been classified as a category 1 human carcinogen (IARC, 1993).

In Senegal, infestation of peanut crops with *Aspergillus* and especially *Aspergillus flavus* is a major national problem, as this crop, which is practiced by 75% of the country's farmers, is both a cash crop and a food crop. It

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contributes 20% of GDP and constitutes more than 30% of food consumption (Clavel et al., 2013). The presence of aflatoxins in groundnut seeds, inherent to this infestation, poses both a public health problem and an economic brake (PACA, 2012).

Composite materials containing silver in the form of a nanometric particle have been very virulent towards micro-organisms (Egger et al., 2009). Thus, we have identified them to supplement existing fungal proliferation control devices in storage locations. To realize the full potential of their antimicrobial properties, however, it is necessary to protect these silver nanoparticles, and the proposed approach is to encapsulate them in an inert layer of silica. The silver@silica nanostructure gives the metal good chemical stability, solves the problems of agglomerations inherent in nanoparticles and also simplifies the connection of nanoparticles to other materials.

MATERIALS AND METHODS

Synthesis of silver@silica nanoparticles

Components of the reverse microemulsion, Triton X-100 (1.77 ml), hexanol (1.8 ml), cyclohexane (7.5 ml) and water (1.84 ml), were introduced at room temperature under stirring in a 30 ml bottle. Then, silver nitrate (15 mg) solubilized in the aqueous phase of the microemulsion was reduced by sodium borohydride (3 mg) in the presence of APTES (0.021 ml). Silver nanoparticles are formed in seconds, as evidenced by the change in color of the microemulsion. After stirring for 30 minutes, tetraethylorthosilicate (0.08ml) and ammonium hydroxide (0.06 ml) were added to the reaction medium to form the silica layer. The obtained mixture was left stirring overnight. Nanoparticles were separated from the reaction medium by addition of an equal volume of ethanol and centrifugation. With supernatant removed, nanoparticles were washed four times with ethanol.

Isolation and identification of an aflatoxigenic *Aspergillus flavus* strain

Peanut seeds collected in the local market were used to isolate *A. flavus*. The seeds of peanuts were deposited on the PDA medium (Potato Dextrose Agar) and incubated at the temperature of 32°C for 3 days. Colonies representative of *A. flavus* were sub-cultured again on to PDA (Samson et al., 2004). Primary identification was made based on cultural characteristics described by Chakranarayan (Chakranarayan et al., 2013) like colony diameter, colony color on agar and reverse and colony texture. In addition, morphological features were studied under the microscope and the major and remarkable microscopic features that were considered were conidiophores, conidial shape,

phialides and metulae, presence and shape of vesicles (Ouattara-Sourabie et al., 2011; Chakranarayan et al., 2013). *Aspergillus* differentiation media (AFPA) is a selective and differential medium which is used for detection of aflatoxin producing *Aspergillus* species. Aflatoxigenic *A. flavus* specie has been distinguished from other *Aspergillus flavus* species based on the reverse orange color on AFPA medium after 72 h of incubation at 32 °C. Orange color in the reverse of the colonies is due to the reaction of ferric ions from ferric citrate present in the medium with the aspergillilic acid molecules synthesized by *Aspergillus* species.

Antifungal activity of silver@silica nanoparticle against aflatoxigenic *Aspergillus flavus*

The technique consists of making a film of nanoparticles on the surface of the culture medium. Four concentrations of silver@silica nanoparticles (2, 3, 4 and 5 mg/ml) were deposited on the surface of the PDA. Each treatment replicated three times with controls (PDA without silver@silica nanoparticle). The efficiency of silver@silica nanoparticles treatment was evaluated after control competed by measuring the fungi colonies diameters. The inhibition rate (%) was calculated by using the following formula: $PI = \frac{Da - Db}{Da} \times 100$

Where Da is the diameter of mycelial growth in control medium and Db is the diameter of mycelial growth in medium treated by silver@silica nanoparticles

Determination of bioactivity type of silver@silica nanoparticles

To know if silver@silica nanoparticles effectively kill *A. flavus* (fungicide effect) or whether silver@silica nanoparticles only allow growth inhibition (fungistatic effect), the Voisin method (Voisin, 2012) was used.

Statistical analysis

The data were analyzed by analysis of variance followed by the Tukey-Kramer (HSD) test at $p < 0.05$ using SPSS software version 16.

RESULTS

Silver@silica nanoparticles

The shape and size of the silver@silica nanoparticles prepared in this study were checked by Transmission Electron Microscopy (TEM). Their structures under electron microscope are shown in Figure 1 and their characteristics are shown in Table 1.

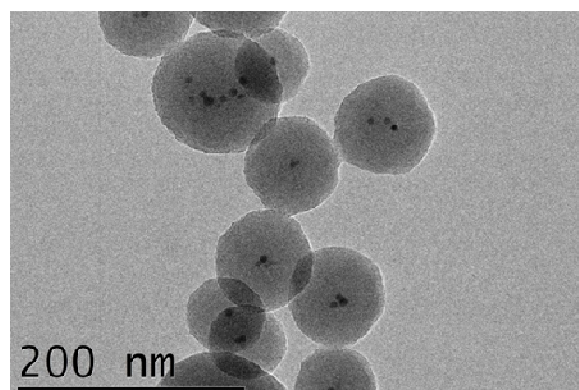


Figure 1. Transmission electron microscopy image of silver@silica nanoparticles

Table 1. Characteristics of silver@silica nanoparticles

Diameter of silver (nm)	Diameter of silica (nm)	Silver weight %
2-11	35-90	10

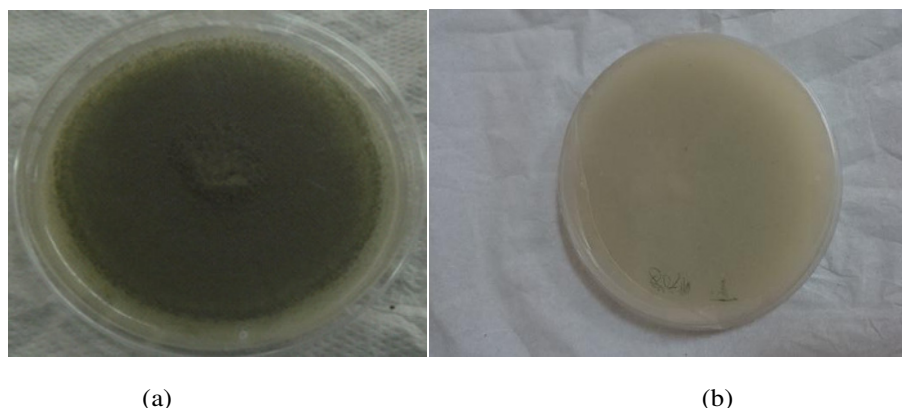


Figure 2. Colony of *Aspergillus flavus* on PDA at 32°C after 7 days of incubation. (a) Front view and (b) Reverse view

Aflatoxigenic *Aspergillus flavus*

The colony obtained was olive green and yellowish on the reverse with a diameter of 9 cm after 7 days of incubation (Figure 2). Microscopic characteristics of *A. flavus* showed globular conidia and a non-partitioned conidiophore, hyaline and colorless. The conidial head was radiant with a globular vesicle (Figure 3). Isolates belonging to *A. flavus* exhibited a bright orange color on the colony reverse of AFPA (Figure 4). This confirmed the aflatoxin producing ability of the *Aspergillus flavus* isolated from peanut samples.

Antifungal activity of silver@silica nanoparticles

The results of the antifungal activity of silver@silica nanoparticles against *A. flavus* were presented in Table 2. It was revealed from the results that the growth of *A. flavus* was significantly reduced by silver@silica nanoparticles. The percent of inhibition increased with concentrations. Between 2 and 4 mg/ml the percent of inhibition ranged from 72.57 % and 93.38 %. However, the percent of inhibition was 100% at 5 mg/ml. Based on the results of the present study, silver@silica nanoparticles have high toxicity on *A. flavus*.

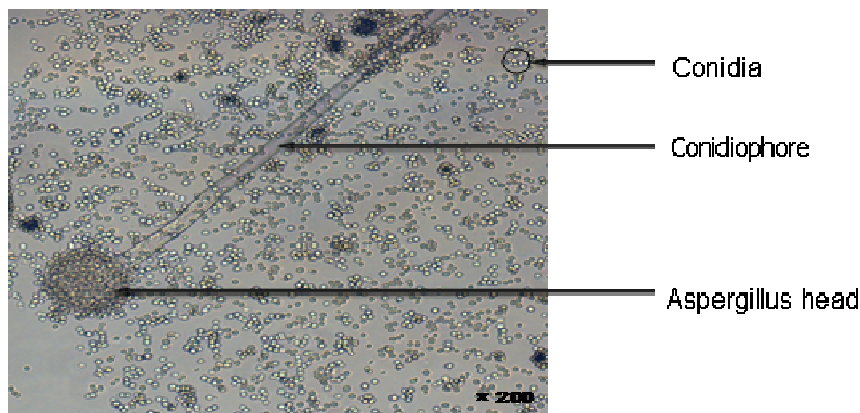


Figure 3. Optical microscopy image of the isolated *Aspergillus flavus*

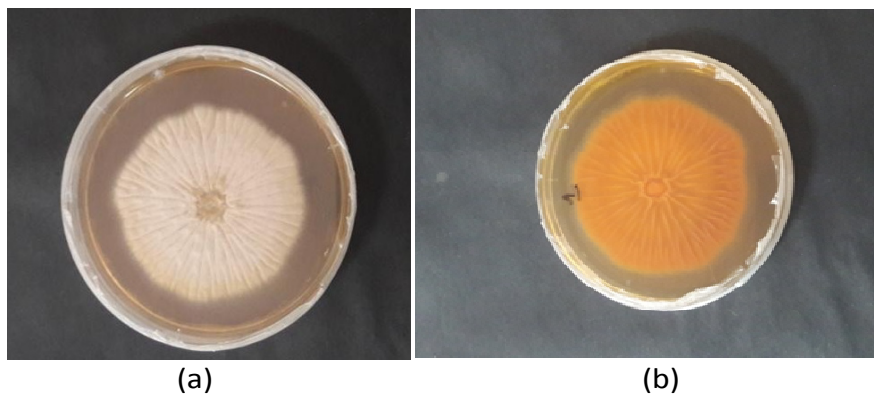


Figure 4. Colony characteristics of aflatoxigenic *A. flavus* on AFPA at 32°C after 7 days of incubation. (a) Colony color: White and (b) Colony Reverse Color: Yellowish orange after 7 days of incubation.

Table 2. Antifungal activity versus silver@silica nanoparticle concentration.

Concentrations (mg/ml)	Inhibition rates (%)
2	72.57 ± 3.86 ^a
3	87.78 ± 2.41 ^b
4	93.38 ± 3.08 ^c
5	100.00 ± 00 ^d

Means followed by the same lowercase letter in each column are not significantly different using Tukey-Kramer test at $p < 0.05$

Bioactivity type of silver@silica nanoparticles

The results showed that silver@silica nanoparticles had fungicide effect to *A. flavus*. The control Petri dish showed a resumption of growth of the strain. However, no

resumption of growth of the mycelium was observed in the Petri dish containing the agar fragment treated with 5 mg/ml of silver@silica nanoparticles (Figure 5).

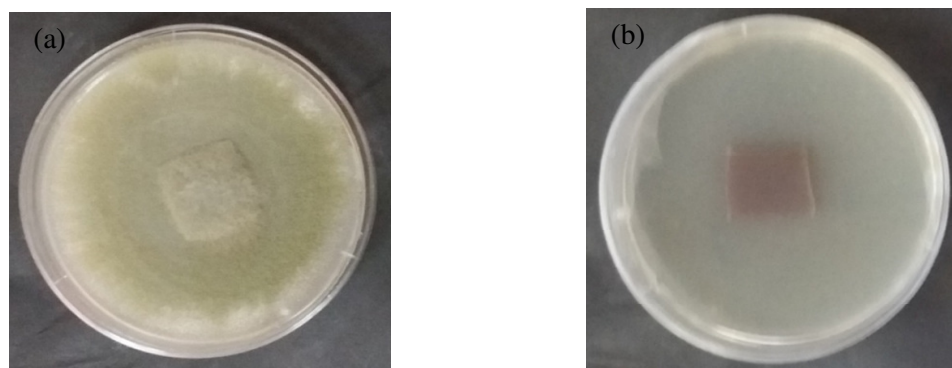


Figure 5. Growth resumption test of *Aspergillus flavus* on PDA at 32°C after 7 days of incubation. (a) Test and (b) control

DISCUSSION

In the present study, the antifungal activity of silver@silica nanoparticles toward aflatoxigenic *A. flavus* was investigated. Silver@silica nanoparticles exhibited a potent antifungal activity against *A. flavus* strain tested. These results indicated that silver@silica nanoparticles have remarkable potential as an antifungal agent to prevent fungal growth and subsequent aflatoxins production. This confirms the concept silver@silica as a reservoir of silver ions that diffuse through the porous silica matrix and inhibit the microbial growth. Similar results have been reported by Acharya et al. (2018) on different bacteria. The antifungal activity of silver@silica nanoparticles depends not only on the amount of silver@silica nanoparticles, but also to the amount of silver encapsulated in the silica matrix. The antifungal effect of the silver@silica nanoparticles could be controlled by varying the percentage of silver encapsulated in the silica matrix.

The antifungal activity of silver@silica nanoparticles is essentially due to the release of silver ions (Zheng et al., 2012; Egger, 2009). The permeability of the silica matrix allows the silver to be oxidized in silver ions which will interact with cellular proteins and DNA. These interactions lead to the inactivation of proteins and create mutation and repress the capacity of DNA replication (Petica et al., 2008; Elechiguerra et al., 2005). Unlike Egger (2009) who used nanocomposites silver /silica in the aggregates form, nanostructure used in this study are more efficient due to the fact that in addition to silver ions, silver@silica nanoparticles strongly interact with the cellular membrane. Silver@silica nanoparticles bind to the fungal cells, accumulate on the outer membrane and then penetrate inside the cell (Salem et al., 2011; Quignard, 2012). Thus more silver ions release inside the fungal cell, that leads to disturbance between other transmembrane energy metabolism (Salem et al., 2011) and ultimately to cell lysis (Elechiguerra et al., 2005; Berger et al., 1976). The interest of silver@silica nanoparticles compared to silver

nanoparticles is that the silica matrix of silver@silica nanostructure gives the silver good chemical stability, solves the problems of agglomerations inherent in silver nanoparticles and also simplifies the connection of nanoparticles to other materials such as coatings.

CONCLUSION

The Study showed that silver@silica nanoparticles have a fungicide effect on aflatoxigenic *A. flavus*. This confirms the concept silver@silica as a reservoir of silver Ag ions that diffuse through the porous silica matrix and inhibit the microbial growth. Silver@silica nanoparticles are therefore presented as a promising alternative to chemicals antifungal products that are very harmful to health and the environment. But as any new technology, investigations have to be done to appreciate the environmental impact and its effect on human and animal health.

ACKNOWLEDGMENTS

This work was supported by the West African Agricultural Productivity Program (WAAP/PPAAO). We thank BecA-ILRI for the internal review of this manuscript.

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