DETECÇÃO DA ATIVIDADE DE NANOPARTÍCULAS DE PRATA, *PSEUDOMONAS FLUORESCENS* E *BACILLUS CIRCULANS* NA INIBIÇÃO DO CRESCIMENTO DE *ASPERGILLUS NIGER* ISOLADO A PARTIR DE FRUTAS ALARGADAS

DETECTING THE ACTIVITY OF SILVER NANOPARTICLES, *PSEUDOMONAS FLUORESCENS* AND *BACILLUS CIRCULANS* ON INHIBITION OF *ASPERGILLUS NIGER* GROWTH ISOLATED FROM MOLDY ORANGE FRUITS

الكشف عن فعالية الجسيمات النانوية للفضة، الزانفة المتألقة و عصوية سيركيولانز على تثبيط نمو الرشاشية السوداء المعزول من ثمار البرتقال المتعفن

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Received 12 March 2020; received in revised form 26 May 2020; accepted 19 July 2020

RESUMO

O acondicionamento e armazenamento inadequados das laranjas (*Citrus sinensis*) pode resultar na deterioração e crescimento de microrganismos, incluindo *Aspergillus* spp. estes fungos são onipresentes e crescem em frutos após a colheita. Certas espécies de *Aspergillus* produzem toxinas que podem afetar a saúde de seres humanos e animais. O presente estudo teve como objetivo isolar *Aspergillus niger* de laranjas mofadas, testar sua capacidade de produzir aflatoxina pelo método Saito e Machida e avaliar sua toxicidade de alguns parâmetros bioquímicos em ratos brancos machos. Além disso, a eficiência da inibição de nanopartículas de prata (AgNPs), *Pseudomonas fluorescens* e *Bacillus circulans* foi avaliada contra isolados de *A. niger*. O resultado da triagem de todos os nove isolados pelo método Saito e Machida demonstrou a capacidade de um isolado produzir aflatoxinas em grandes quantidades. O filtrado desse isolado mostrou um aumento no nível de enzimas GPT e GOT (24,2 U/ml e 18,3 U/ml), respectivamente, em comparação com o isolado de aflatoxinas não produtoras e o controle (16,6 e 12,2 U/ml) e (8,5 e 8,2 U/ml), respectivamente. O nível de glicose foi aumentado para (130,2 mg/dl) nos ratos tratados com o filtrado do isolado produtor de aflatoxinas em comparação com (95 mg/dl) e (86,4 mg/dl) tratados com o filtrado do isolado não produtor e o controle respectivamente. Além disso, o nível de LDL-colesterol foi reduzido para 120,2 mg/dl e 101 mg/dl com o produtor de filtrado e os isolados de aflatoxinas não produtoras, respectivamente, em comparação com 144,3 mg/dl para o controle. Os AgNPs mostraram um efeito inibitório contra o crescimento de *A. niger*, com a maior zona de inibição de 28,38 mm a uma concentração de 100 µl. Por outro lado, *Pseudomonas fluorescens* e *Bacillus circulans* também mostraram uma excelente taxa de inibição (100%) na concentração de 2 g/L do filtrado do fungo. Esses resultados expuseram que os AgNPs são capazes de inibir *A. niger* e, consequentemente, os AgNPs podem ser usados como um candidato antifúngico.

Palavras-chave: *Citrus sinensis*, *Aspergillus niger*, Parâmetros bioquímicos, In vitro, Nanopartículas de prata.

ABSTRACT

The improper packaging and storage of oranges (*Citrus sinensis*) may result in decay it and growth of microorganisms, including *Aspergillus* spp. these fungi are omnipresent and growing on post-harvest fruits. Certain species of *Aspergillus* produce toxins that can affect humans and animals health. This study aimed to isolate *Aspergillus niger* from moldy oranges, test its ability to produce aflatoxin using the Saito and Machida method, and evaluation of their toxicity of some biochemical parameters in white rat males. Moreover, the inhibition efficiency of silver nanoparticles (AgNPs), *Pseudomonas fluorescens*, and *Bacillus circulans* were assessed against *A. niger* isolates. The outcome of screening all nine isolates by the Saito and Machida method has shown the capability of one isolate to produce aflatoxins in large amounts. The filtrate of this isolate showed an increase in the level of GPT and GOT enzymes (24.2 U/ml and 18.3 U/ml) respectively, compared to the

non-producing aflatoxins isolate and the control (16.6 and 12.2 U/ml) and (8.5 and 8.2 U/ml) respectively. Glucose level was increased to (130.2 mg/dl) in the rats treated with the filtrate of aflatoxins producer isolate compared to (95 mg/dl) and (86.4 mg/dl) treated with the filtrate of the non-producer isolate and the control respectively. Additionally, the LDL-cholesterol level was reduced to 120.2 mg/dl, and 101 mg/dl with the filtrate producer and the non-producer aflatoxins isolates respectively compared to 144.3 mg/dl for the control. AgNPs have shown an inhibitory effect against *A. niger* growth with the largest inhibition zone of 28.38 mm at a concentration of 100µl was recorded. On the other hand, *Pseudomonas fluorescens* and *Bacillus circulans* have also shown an excellent inhibition rate (100%) at the concentration of 2 g/L of the fungus filtrate. These results exposed that AgNPs are able to inhibit *A. niger*, and consequently, AgNPs can be used as an antifungal candidate.

Keywords: *Citrus sinensis*, *Aspergillus niger*, Biochemical parameters, In vitro, Silver nanoparticles.

المخلص

التغليب والتخزين غير المناسبين للبرتقال (*Citrus sinensis*) قد يؤدي الى تحلله ونمو الكائنات الحية الدقيقة بما في ذلك نوع الرشاشيات هذه الفطريات منتشرة في كل مكان وتنمو على فاكهة ما بعد الحصاد. تنتج بعض انواع الرشاشيات السموم التي يمكن أن تؤثر على صحة الإنسان والحيوان. تهدف هذه الدراسة إلى عزل فطريات الرشاشية السوداء من البرتقال المتعفن، اختبار قدرتها على إنتاج الأفلاتوكسين باستخدام طريقة Saito و Machida، وتقييم سميتها لبعض المعايير الكيموحيوية في ذكور الجرذان البيضاء. علاوة على ذلك، تم تقييم كفاءة تثبيط الجسيمات النانوية للفضة، بكتيريا الزائفة المتألقة و عصوية سيركيولانز ضد عزلات فطر الرشاشية السوداء. أظهرت نتائج مسح جميع العزلات التسعة بطريقة Saito و Machida قدرة عزلة واحدة على إنتاج الأفلاتوكسين بكميات كبيرة. أظهر راشح هذه العزلة زيادة في مستوى إنزيمات GOT و GPT (24.2 وحدة/مل و 18.3 وحدة/مل) على التوالي، مقارنة بالعزلة غير المنتجة للأفلاتوكسين ومعامل الضبط (16.6 و 12.2 وحدة/مل) و (8.5 و 8.2 وحدة/مل) على التوالي. مستوى الجلوكوز ازداد إلى (130.2 مجم/ديسيلتر) في الجرذان المعاملة براشح العزلة المنتجة الأفلاتوكسين مقارنة بـ (95 مجم/ديسيلتر) و (86.4 مجم/ديسيلتر) المعاملة براشح العزلة غير المنتجة والمجموعة الضابطة على التوالي. بالإضافة إلى ذلك، انخفض مستوى الكوليسترول الضار إلى 120.2 مجم/ديسيلتر و 101 مجم/ديسيلتر المعاملة مع راشح العزلات المنتجة وغير المنتجة للأفلاتوكسين على التوالي مقارنة بـ 144.3 مجم/ديسيلتر للمجموعة الضابطة. أظهرت الجسيمات النانوية للفضة تأثير مثبط ضد نمو فطر الرشاشية السوداء مع أكبر منطقة تثبيط 28.38 ملم بتركيز 100 ميكرو لتر. من ناحية أخرى، أظهرت أيضا كل من بكتيريا الزائفة المتألقة و عصوية سيركيولانز معدل تثبيط ممتاز (100 %) عند تركيز 2 جم/لتر من راشح الفطر. كشفت هذه النتائج أن الجسيمات النانوية للفضة قادرة على تثبيط فطر الرشاشية السوداء وبالتالي يمكن ترشيحها لاستخدامها كمضاد للفطريات.

الكلمات المفتاحية: البرتقال، فطر الرشاشية السوداء، المعايير الكيموحيوية، خارج الجسم، جسيمات الفضة النانوية.

1. INTRODUCTION

Orange (*Citrus sinensis*) is a type of citrus fruit that belongs to the family Rutaceae. It is one of the winter fruits with an excellent source of vitamin C, in addition to some other vitamins such as B1, B2, B3, B5, B6, A, and E. Furthermore, many other essential nutrients are found in orange, for example, the essential potassium, calcium, iron, zinc, and phosphorus (Butu and Rodino, 2019). Orange is very rich in natural antioxidants, and phenolic compounds, being an important source of flavanones, particularly hesperidin and naringenin. In addition to the hydroxycinnamic acids, orange has a high content of carotenoids, which is an important dietary source of vitamin A carotenoids (β -carotene, α -carotene, and β -cryptoxanthin) and antioxidant carotenoids (β -carotene, β -cryptoxanthin, zeaxanthin, and lutein) and numerous volatile organic compounds producing orange aroma, including aldehydes, esters, terpenes, alcohols, and ketones (Perez-Cacho and Rouseff, 2008; Galaverna and Dall'Asta, 2014; Aschoff et al., 2015; Tamokou et al., 2017; Capurso et al., 2018). Orange peel is plentiful in flavonoids, including methylated derivatives such

as PMFs, which exhibited strong anti-inflammatory effects at both gene expression and enzyme activity levels (Yu et al., 2004; Sandhy et al., 2011; Manthey et al., 1999).

Previous studies have shown that 20% of processed fruits and vegetables are lost due to spoilage (Mailafia et al., 2017). Oranges withstand at room temperature for long enough but can last up to three weeks if kept in the refrigerator (Butu and Rodino, 2019).

The improper handling, packaging, warehousing, and transportation may result in decomposition and growth of microorganisms. The decay becomes activated because of changes in the physiological state of the fruits, as the pH level becomes lower, in addition to any other reasons, for instance, the high moisture content and the high nutrient composition which both assist in making the fruits very susceptible to attack by pathogenic fungi, which at the end causing rot, and may also make the fruits unfit for consumption by producing mycotoxins (Moss, 2002; Jay, 2003). Common air molds such as *Penicillium* and *Aspergillus* species may enter into the tissues and causing loss during packaging. The extensive production of spores by these pathogens allows it to maintain its

presence wherever fruits were handled, including field, packinghouse, equipment, storage rooms, transit containers, and market place (Ismail and Zhang, 2004).

Aspergillus niger is a parasitic fungus that penetrates the tissues of citrus fruits through micro-wounds and bruises. *Aspergillus* rot has a black mold on the fruits, and even adjacent fruits are infected as the spores contaminate the whole lot. High temperatures (the most favorable temperature is 25–40 °C) and high relative humidity support these organisms to develop. *Aspergillus niger* causes rapid decay and spreads at 30–35 °C very quickly. *Aspergillus* does not get below 15 °C, and in that respect is no increase at all under refrigerated conditions. The fungus is very sensitive to temperature. Interestingly, acid lime fruit stored at 30–35 °C develops very high rates of decay and sporulation when infected, while fruits from the same lot stored under cooled conditions (8–10 °C) is free of decay. *Aspergillus niger* is increasingly widespread in Indian citrus fruits (Milind, 2008).

Nanoparticles are one of the materials that reflect an excessive interest due to their properties that make them an excellent candidate for numerous castigations such as medicine, biology, bioengineering, and much more. Silver, copper, gold, magnesium, titanium has been used as nanoparticles against different species of microbes. However, the silver nanoparticles are presently interesting, and the attention to this metal is increased to be used in many uses, including antifungal agents (Gong *et al.*, 2007; Ahmad *et al.*, 2006; Aldujaili and Banoon, 2020).

Silver nanoparticles (AgNPs) are metal structures at the nanoscale and the most important choice for solving various medical problems due to their biocompatible chemical structure, inertness, oxidation resistance, and the broad spectrum of antimicrobial activity against a diverse range of bacteria and fungi. Additionally, silver nanoparticles have very little chance of developing microbial strains resistant to drugs (Mallmann *et al.*, 2015). Silver nanoparticles, therefore, have synthesized as potential antimicrobial material against fungi and bacteria to treat many of the infectious diseases caused by human pathogens and to eliminate the multidrug resistance problems (Prabhu and Poulouse, 2012; Salem *et al.*, 2015; Gudikandula *et al.*, 2017).

This study aimed to isolate *A. niger* from fruits of moldy oranges, test there ability to produce aflatoxins using the Saito and Machida

method, and evaluation the toxicity of *A. niger* on some biochemical parameters in white rat males and assessment the inhibition efficiency of silver nanoparticles, *Pseudomonas fluorescens* and *Bacillus circulans* against *A. niger* isolate that produced the aflatoxins.

2. MATERIALS AND METHODS

2.1 Bacterial model species used in this study:

Pseudomonas fluorescens and *Bacillus circulans* were used in this study. Both strains were identified and confirmed by applying the biochemical tests and using the VITEK-2 compact system using GP and BCL cards, respectively (Garrrity *et al.*, 2004).

2.2 *Aspergillus niger* isolated from decayed orange fruits

Aspergillus niger was isolated from moldy orange fruits which were surface disinfected with 4% sodium hypochlorite for 2 min, the surface of disinfected fruits were plated on sterilized Petri dishes contained Potato Dextrose Agar (PDA) which was prepared according to (Mackie 1996). The plates were incubated at 28 °C for seven days. The identification of *Aspergillus niger* isolates was based on cell and colony morphology characteristics according to the method described by (Klich 2002; Samuel *et al.*, 2015). At the final stage of the incubation period, the percent of the appearance of fungus was calculated by Equation no.1.

2.3 Detection of the capacity of *Aspergillus niger* isolates to produce aflatoxins

Nine isolates of *A. niger* were obtained from a total of 15 fungal samples. The ability of these isolates to produce the aflatoxins was tested according to (Saito and Machida, 1999) on Coconut Extract Agar (CEA) medium and ammonia solution at a concentration of 20%. The plates were incubated at 28 °C for 7-14 days. The CEA medium was prepared according to (Alvarez-Barrea *et al.*, 1982).

2.4 The effect of the *A. niger* Filtrate on white rat males

2.4.1 Preparation of Fungal Filtrate

Three flasks (500 ml) were prepared, and 250 ml from liquid yeast extract medium was

added to each flask. After the sterilization and cooling of the medium, two discs of *A. niger* isolate that produced the aflatoxins were added into the first flask, two discs of *A. niger* isolate that does not produce the aflatoxins were added into the second flask. The third flask that does not contain any fungi isolates was used as a control treatment. All three flasks were incubated at 25 ± 2 °C for three weeks. After the incubation period, the components of the fungus (fungus and spores) were separated using turmeric papers (Whatman NO. 4) with the Buchner suppression. The fungal filtrate for both isolates were then collected and used in the dosages of animals (Al-Janabi, and Al-gumaili, 2009).

2.4.2 Preparation of Laboratory animals

In this experiment, 15 white male rats (10 weeks of age, average weight: 200 g) were tested. They were obtained from the University of Kufa, Najaf City, Iraq. The rats were acclimatized for three weeks and housed in polyethylene boxes with bedding of wood shavings. They were maintained under standard conditions, which included a temperature of approximately 22-24 °C with a regular 12h light/12h dark cycle. All rats were given standard rodent pellet food and water. These rats were separated into three groups. Each group includes five rats:

Group A: 5 rats were orally injected with *A. niger* filtrate produced the aflatoxins and in a dose of (1 ml/ 100 g /day) per body weight.

Group B: 5 rats were orally injected with *A. niger* filtrate non-produced the aflatoxins at the same concentration and method mentioned in group A.

Group C: 5 rats were treated with filtrate of the third flask that does not contain any fungi isolates, and the same concentration and method mentioned in group A, treated as a control.

The dosage was taken every 48 hours by oral gavage (Waynforth and Flecknall, 1980), after three weeks of the experiment, the animals were left for three days, the animals were anesthetized and sacrificed.

2.5 Biochemical parameters

The biochemical parameters, including the determination of the GOT and GPT levels, determination of glucose level in serum, and estimate the level of cholesterol in the blood, were carried out according to the manufacturer's instructions (Linear Chemical, Spain).

2. 6 Preparation of Silver Nanoparticles

Silver nanoparticles powder (Shanghai Ximeng New Materials Technology co., Ltd, China) Type 2, Purity > 99.95%, with particle size (50nm) was used, The aqueous solution of silver nanoparticles was prepared by dissolving 20 mg of AgNPs in 100 ml of deionized water. The resulted solution was stirred well by the sonication process to obtain a homogeneous solution and to avoid the accumulation of the nanoparticles. This mixture was then autoclaved at 15 psi pressure, 121 °C for 5 minutes. The solution was yellowish, the serial dilution concentrations of silver nanoparticles were required in this study prepared 25, 50, 75, and 100 (μl) (Mohanty *et al.*, 2012).

2.6 Antifungal activity of the silver nanoparticles

The procedure described by (Alananbeh *et al.*, 2017) was followed here with some modification; by preparation of the PDA medium. The PDA was sterilized in the autoclave and poured into 6 Petri dishes. The dishes were then inoculated with *A. niger* isolate, which produced the aflatoxin. The fungicidal activity of the silver nanoparticles was determined using the agar well diffusion assay method. All the dishes were incubated at 25 ± 2 °C for five days, and the plates were examined for evidence of zone of inhibition, which appeared as a clear area around the wells, the diameter of such zones of inhibition was measured using a meter ruler.

2. 7 Antifungal activity for the bacteria used in this study

The PDA was prepared and distributed in three flasks at 250 ml per flask. The growth medium was autoclaved for 15 min, after cooling the flasks, the bacterial concentrations (1, 2) g/L were added. The contents of each flask were poured into six dishes, and all dishes were incubated at 25 ± 2 °C from 5 to 7 days (Leben *et al.*, 1987). The dishes were vaccinated with *A. niger* isolate produced the aflatoxins, but the content of the third flask used without bacteria as control. The dishes were then incubated at 25 ± 2 °C for a week. The percentage inhibition of growth of the fungus was calculated using Equation 2 (Kra *et al.*, 2009). In Equation no. 2, A, and B are the average diameter of fungal growth on the control medium with and without bacteria, respectively.

2.8 Statistical Analysis

The obtained data were statistically analyzed using the software SPSS Version 6. Treatment averages were compared at the $p \leq 0.05$ level of probability using LSD (Norris, 1999).

3. RESULTS AND DISCUSSION

3.1 Isolation of Fungi from the orange fruits

Nine isolates were obtained from *A. niger* with an occurrence of 60%. Also, five isolates of *A. flavus* were obtained with 33.33%, while one isolate from *Rhizopus stolonifer* was obtained with 6.66% (Figure 1). These results are close to the results reported by (Reuther, 1967), who confirmed the ability of *A. niger*, *A. flavus*, and *Rhizopus* to infect orange and apple fruits after harvesting. *A. niger* has the highest frequency since it is considered as one of the widespread fungi in different soils, and it can tolerate drought and a wide range of wet tension (Hillocks and Waller, 1997). Also, *A. niger* spores have been found to withstand at high temperatures (up to 50 °C) (Gabler *et al.*, 2004). The present study agreed with (Mailafia *et al.*, 2017), the authors found that *Aspergillus niger* had the highest occurrence in pineapple, watermelon, oranges, papaw, and tomatoes with a frequency of 38%. The percentage of *Rhizopus* as the lowest agree with (Muhammad *et al.*, 2018) found the *Rhizopus* spp (20%), according to other fungi that isolated from sweet orange.

Figure 1. The frequency rate of isolated fungi from orange fruits

3.2 Detection of the ability of *A. niger* isolates to produce aflatoxins

The results of the chemical detection using the coconut medium and ammonia have shown that some of the *A. niger* isolates were able to produce aflatoxins (Table 1). It was noticed that the isolate no. 2 produced large

quantities of aflatoxins while isolates no.5 and no. 8 produced small quantities of aflatoxins, other isolates were negative for aflatoxin production. The difference in the ability of the isolates to produce aflatoxins, as mentioned by (Saito and Machida, 1999), depends on the degree of red color, the dark red indicates its ability to produce larger quantities of aflatoxins, and this result agrees with (Scott and Trucksess, 1997) who confirmed the ability of *Aspergillus niger*, *Aspergillus ruber*, *Aspergillus wentii*, and *Aspergillus ostianus* to produce aflatoxins but in lower quantities of *Aspergillus flavus*. Also (Hasan, 2000) reported that some of the *A.niger* isolates that were isolated from apple fruits could produce aflatoxins. A study by (Akinola *et al.*, 2019) reported that twenty-three isolates from *Aspergillus* were positive for aflatoxin production. Some *A. niger* strains have been reported to produce potent mycotoxins called ochratoxins, which can be harmful to humans and animals (Mailafia *et al.*, 2017).

Table 1. *Aspergillus niger* isolates producing and non-producing the aflatoxins

No. of <i>niger</i> isolates	A. Quantities Aflatoxins	The ability of aflatoxins production
An1	-	-
An2	Large	++
An3	-	-
An4	-	-
An5	Small	+
An6	-	-
An7	-	-
An8	Small	+
An9	-	-

An2: *A.niger* isolate that produced aflatoxins in large amount, and it used in this study

An6: *A.niger* isolate that non-producing of aflatoxins, and it used in this study

3.3 The toxic effects of the *A. niger* Filtrate on white rat males

3.3.1 Effect on GPT and GOT enzymes

Figures (2 and 3) show the effect of two isolates of *A. niger* on GPT and GOT enzymes. The *A. niger* isolate that produced the aflatoxins increased the activity of the GPT and GOT to 24.2 U/ml and 18.3 U/ml, respectively. In

contrast, the *A. niger* isolate that non-produced the aflatoxins increased the activity of two enzymes to 16.6 U/ml and 12.2 U/ml, respectively, compared to the control treatment of 8.5 and 8.2 U/ml.

The increase in the activity of enzymes in the serum of treated animals can be explained by the fungal filtrate that may have an effect on the liver cells containing these enzymes, which leads to releasing the enzymes to the blood. This mechanism can also affect other organs such as kidneys and membranes of the endoplasmic network, resulting in disruption of the bio-transport system in the body (Meerdink, 2004). This study agrees with (Al- Janabi and Al-gumaili, 2009), who showed that the isolate of *A. flavus*, which produces aflatoxins, significantly increased the level of GPT and GOT to 22.3 and 14.4 U/ml compared to the control treatment of 8.3 and 8.2 U/ml respectively.

Figure 2. Effect of *A. niger* isolates on GPT, A: aflatoxins production isolate B: aflatoxins non-production isolate C: control

Figure 3. Effect of *A. niger* isolates on GOT, A: aflatoxins production isolate B: aflatoxins non-production isolate C: control

3.3.2 Effect on Glucose and cholesterol level

The Table (2) shows the results of the effect of the fungal filtrate of *A. niger* isolate on the glucose level, which had caused an increase in the glucose level to (130.2 mg/dl) in the animals that treated with the *A. niger* isolate which producing the aflatoxins and to (95 mg/dl) with the non-producing isolate compared to the

control which was 86.4 mg/dl.

Mechanisms of increasing of the concentration of glucose during aflatoxicosis are not well understood, but this may be due to hormone alteration or stress condition that occurs during aflatoxicosis, which is known to raise blood sugar by altering insulin action. Hyperglycemia in the presence of cirrhosis and some other liver disease may be greater in degree and more prolonged (Yeung and Wang, 1974). The reason of the high sugar level may be due to the metabolic products of the *A. niger* that is normally interfered with the metabolic pathway of glucose and influences the enzymes responsible for regulating the amount of sugar in the blood, including insulin, this is indicated by (WHO, 2003; Sacks *et al.*, 2002). A study by (Mor *et al.*, 2017) reported that Ochratoxin A from *Aspergillus* leads to decreases in insulin levels and increases in blood glucagon and glucose levels. This phenomenon, due to the Ochratoxin A, cause pancreatic damage on the Langerhans islet cells and predispose rats to diabetic Mellitus.

The blood glucose decrease during the course of aflatoxicosis, then after 30 days of treatment, blood glucose levels slowly increased, as indicated by decreasing the concentration of glycogen in the liver, kidney, and heart, which may result from the reducing tissue glucose intake (Busby and Wogan, 1981; Kolhe, 2016).

For the LDL cholesterol, the filtrate of *A. niger* isolate that produced aflatoxins reduced the cholesterol level to 120.2 mg/dl while the non-productive isolate reduced to 101 mg/dl compared to the control with a cholesterol level of 144.3 mg/dl. Hussein and Brasel (2001); Pusztahelyi *et al.* (2015), noted that fungal secondary metabolic products contain compounds that have a high affinity for the mitochondrial membrane causing a rapid crash and also inhibit the protein transport chain, this inhibition may lead to a reducing in the production of energy that required to synthesize cholesterol. Also, Javed *et al.* (2014) found that seven indigenously of *Aspergillus* strains were able to produce cholesterol-lowering drug lovastatin, and the *Aspergillus terreus* strain showed maximum production, the optimized lovastatin was extracted from fermented broth and orally administered to rats. It was concluded that fermented lovastatin effectively lowers the cholesterol level of rats, while Rashid *et al.* (2013) found that the highest lovastatin production was produced by *Aspergillus niger* SAR strain.

Table 2. Effect of *A. niger* isolates that produced and non-produced of aflatoxins in the level of sugar and cholesterol in white male rats

Type of treatment	Blood sugar level (mg/dl)	Blood cholesterol level (mg/dl)
<i>A. niger</i> isolate that produced of aflatoxins	130.2	120.2
<i>A. niger</i> isolate that not produced of aflatoxins	95	101
control	86.4	144.3
L.S.D(0.05)	0.34	0.16

Note: Each number represents three replicates.

3.4 The efficiency of silver nanoparticles in the inhibition of *A.niger* radial growth

The results indicated that the silver nanoparticles were able to inhibit *A. niger* growth. The diameters of the inhibition zone (11.51, 16.28, 23.71, 28.38 mm) at concentrations 25, 50, 75, and 100 (µl), respectively, as shown in Figure 4. Previous studies described the effective applications of silver nanoparticles as an inhibitory agent at different concentrations against fungi such as *Alternaria alternate*, *Fusarium*, *Aspergillus* species, *Trichophyton mentagrophytes*, and *Candida* sp. (Gao *et al.*, 2012; Noorbakhsh *et al.*, 2011; Bocate *et al.*, 2019). Also, a study by (Alananbeh *et al.*, 2017) found that the gradual reduction in growth was clear in both *Aspergillus* species as the silver nanoparticles concentrations were increased, where *A. terreus* had higher reduction compared to *A. niger*. The silver nanoparticles are possible to be used as antifungal substances. The AgNPs mode of action has several explanations. Metal depletion is one of the AgNP's modes of action that forms irregularly shaped pits in the outer membrane and changes membrane permeability caused by the progressive release of molecules and membrane proteins from lipopolysaccharides (Amro *et al.*, 2000). Ag-generated free radicals have been documented using the Ag nanoparticles Electron Spin Resonance (ESR). Ag nanoparticles' antimicrobial function has to do with the creation of free radicals and subsequent free radical-induced damage to the membrane (Danilczuk *et al.*, 2006). Toxicity is concentration-

and size-dependent. Moreover, the effectiveness of AgNPs against microbes depends on their shapes and sizes (Panáček *et al.*, 2009). In this study, 50nm was the diameter of the AgNPs, yet it inhibited the *Aspergillus niger*. This could be explained by the fact that larger particles of AgNPs can persist longer and could serve as continuous Ag ions source Dobias and Bernier-Latmani (2013), and this agrees with Alananbeh *et al.* (2017).

3.5 The efficacy of *Pseudomonas fluorescens* in inhibition of the radial growth of *A. niger*

The results showed the ability of the *P. fluorescens* to inhibit the growth of *A.niger* isolate that produced aflatoxins with inhibition rate attained 100% at 2 g/L, as shown in figure (5). These results are consistent with many studies such as (Hassan, 2012), the inhibitory capacity of *P. fluorescens* to their ability to produce multiple types of antibiotics including Oligomycine, Oomycine, Phenazine-1-carboxylic acid (PCA), Pyoluterin (Plt) and Pymolnitrin (Pln) that the most important antibiotics produced by *P. fluorescens* in contrast with *Fusarium graminearum* and *Fusarium moniliforme* (Pal *et al.*, 2001). Also, a study conducted by (Mathre *et al.*, 2003), it was reported that *P. fluorescens* was capable of producing 2,4-Diacetylphloroglucinol 2,4-DPG), which inhibits the growth of many fungi-causing diseases.

3.6 The efficacy of *Bacillus circulans* with different concentrations in inhibition of the radial growth of *A.niger*

Bacillus circulans proved highly effective in inhibiting the growth of *A. niger* isolate that producing aflatoxins with an inhibition rate attained 100% at 2 g/L, as shown in Figure 6. The effect of *B. circulans* may occur due to the mechanisms that bacteria possess against pathogenic fungi, including *A. niger*. These mechanisms include the production of antifungal compounds, many of these compounds, such as A Zwittermicin, Kanosamine, and Bacillomycin. These compounds affect fungal cell components, such as enzymes and proteins (McSpadden Gardener, 2004).

Bacillus species can also produce some enzymes that analyze cellular walls of fungus such as chitinase, which dissolves the substance of chitin, the base component of the cellular walls of fungus (Peraica *et al.*, 1999). Also (Idriss *et al.*, 2002) demonstrated the ability of some species of *Bacillus* to produce the phytase that broken of

Phytic acid in the cell walls of many fungi.

4. CONCLUSIONS

The outcomes of the present study found that one isolate of *A.f A. niger* was able to produce aflatoxins by using the Saito and Machida method.

The isolate of *A. niger* that was producing aflatoxins, which effect on GPT and GOT enzymes that cause increased the level of the two enzymes compared to the isolate of *A. niger* that non-producing aflatoxins and the control treatment.

The results exposed that the silver nanoparticles were able to inhibit *A.niger* isolates growth at concentrations of 25, 50, 75, and 100 (µl). AgNPs can be used as antifungal substances. They considered as less harmful and cost-efficient than other methods for preparing the Nanoparticles.

The results also showed the efficacy of *Pseudomonas fluorescens*, and *Bacillus circulans* in the inhibition of the *A. niger* isolates growth when they used in the concentration of 2 g/L.

5. ACKNOWLEDGMENTS:

Authors thank the Department of Biology, Faculty of Science, University of Kufa, and the University of Misan for providing the facilities to conduct this study.

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$$\text{The percentage of appearance} = \frac{\text{number of samples in which the fungus appeared}}{\text{The total number of samples}} \times 100 \quad (\text{Eq. 1})$$

$$\text{percentage inhibition} = \frac{(A-B)}{A} \times 100 \quad (\text{Eq. 2})$$

Figure 4. The capacity of silver nanoparticles in inhibition of the radial growth of *A. niger*: A. Effect of different concentrations of silver nanoparticles B. The control treatment.

Figure 5. The efficacy of *P. fluorescens* in inhibition of the radial growth of *A. niger* isolate that producing aflatoxins, A: The *P. fluorescens* treatment in the concentration of 2g/L, B: The control treatment.

Figure 6. The efficacy of *B. circulans* in inhibition of the radial growth of *A. niger* isolate that producing aflatoxins, A: The *B. circulans* treatment in the concentration of 2g/L, B: The control treatment.