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The *In vitro* Effect of Berberine Sulphate and Berberine Chloride on the Growth and Aflatoxin Production by *Aspergillus flavus* and *Aspergillus parasiticus*

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Abstract

Background: Aflatoxins are harmful mycotoxins that can contaminate animal feed and food products. Plant compounds have been explored as potential agents to inhibit the growth and aflatoxin production of toxigenic fungi.

Objectives: This study aimed to evaluate the in vitro effect of berberine sulphate and berberine chloride on the growth and aflatoxin production of *Aspergillus flavus* and *A. parasiticus.*

Methods: The antifungal activity of berberine salts was determined according to the Clinical and Laboratory Standards Institute (CLSI) document M38-A3. The aflatoxin levels were measured using High Performance Liquid Chromatography (HPLC) method.

Results: The berberine sulphate and berberine chloride showed inhibitory effects against both *Aspergillus* species, with MICs ranging from 125 to 500 µg/ml. Berberine sulphate at 2000 µg/ml and berberine chloride at 1000 µg/ml completely inhibited the mycelial growth of *A. flavus*, while berberine chloride at 1000 µg/ml also completely inhibited the mycelial growth of *A. parasiticus*. Berberine sulphate at 2000 µg/ml reduced the mycelial growth of *A. parasiticus* by 96.7%.

Conclusion: Berberine salts significantly decreased the total aflatoxin production by both *Aspergillus* species at MIC/2 and MIC/4 concentrations ($P< 0.05$). The results suggest that

berberine salts could be used as potential antifungal and antiaflatoxigenic agents against toxigenic *Aspergillus* isolates.

Keywords: Aflatoxins, *Aspergillus flavus*, *Aspergillus parasiticus*, Berberine, Mycelial growth.

Introduction

In the last few decades, the global trade of plant products such as grains, flours, and oilseeds has grown significantly, and for this reason, the contamination of these products with various chemical compounds, especially mycotoxins, has become an important global issue (Moretti *et al*., 2017; Santos Pereira, 2019). By growing on food products, fungi not only lead to a decrease in the nutritional value of these products, but also severely affect the quality of these products by producing mycotoxins (Vieira, 2003).

Contamination of feed and its primary items with mycotoxins may occur before harvest in the field due to the growth of pathogenic fungi on the plant or during the processing and storage of products due to the growth of saprophytic fungi (Gruber- Dorninger *et al*., 2019). Mycotoxins are secondary metabolites produced by many species of fungi. Aflatoxins are a group of mycotoxins that are mainly produced by different species of the genus *Aspergillus*, in particular *Aspergillus flavus* and *A. parasiticus*, after harvest, during storage and processing (Nakavuma *et al*., 2020; Khorrami *et al*., 2022). So far, more than 20 metabolites of aflatoxins have been identified, but only 4 metabolites B1, B2, G1 and G2 are capable of poisoning humans and

animals (Santos Pereira *et al*., 2019). Among the harmful effects of these toxins on humans and animals, we can mention carcinogenesis, mutagenicity, weakening of the immune system, and liver and kidney poisoning (Nakavuma *et al*., 2020; Jard *et al*., 2011; Monson *et al*., 2015; Al-Mudallal, 2023; Mokhtari Hooyeh *et al*., 2022). The prevention of food contamination with aflatoxins is primarily based on preventing the contamination of these products with fungal spores and then controlling the storage conditions such as temperature, humidity and the use of antifungal gas compounds. Another approach proposed to prevent food contamination by fungi is the use of additives that prevent the mycelium growth of toxin-producing fungi and inhibit or reduce the production of aflatoxin by them (Gruber- Dorninger *et al*., 2019; Patil *et al*., 2014; Kadium *et al*., 2023). In recent years, the use of chemical fungicides has faced restrictions due to the health risks for humans and animals and the emergence of resistance to them. Therefore, the use of plant compounds with antifungal properties and preventing the production of aflatoxins has received a lot of attention (Hu *et al*., 2017; Hasankhani *et al*., 2023).

Plants have a wide range of herbal compounds with therapeutic and biological properties. These compounds are mainly classified as alkaloids, flavonoids, tannins, terpenoids and steroids and have been widely used as medicine and additives by humans throughout history (Savoia, 2012). Berberine, a naturally occurring benzyl isoquinoline alkaloid, found in the roots, rhizomes, and stem bark of natural herbs, such as *Berberis aquifolium*, *B. vulgaris* and *B. aristata* (Ghavipanje *et al*., 2022). Berberine has been used for more than 3000 years in the

traditional medicine of Iran and China as an herbal compound with many therapeutic properties against Alzheimer's, Parkinson's, cancer, obesity and diabetes. Also, this composition has antiviral, bacterial and fungal properties (Arayne *et al*., 2007). Berberine and its derivatives have inhibitory effects on the growth and production of toxins by fungi, and so far this effect has been identified in *Candida*, *Fusarium*, *Penicillium* and *Aspergillus* species (Da Silva *et al*, 2016; Ismail *et al*., 2020; El- Zahar *et al*., 2022). Recently, various studies have been conducted to evaluate this isoquinoline alkaloid as a natural preservative with significant antioxidant and antimicrobial properties (Geerlofs *et al*., 2019; Malekinezhad *et al*., 2021). So far, limited studies have investigated the effect of berberine on the growth and mycotoxin production by fungi. Therefore, this study aimed to evaluate the effect of berberine sulfate and berberine chloride on the growth and aflatoxin production by *A. flavus* and *A. parasiticus*.

Materials and Methods

Fungal strains

A frozen stock of *A. flavus* (ATCC 28539) and *A. parasiticus* (ATCC 15517) were obtained from the fungal collection of the Department of Mycology, Faculty of Veterinary Medicine, University of Tehran, Iran.

Berberine salts

Berberine chloride and berberine sulfate were purchased from Sigma company (Sigma-Aldrich, St. Louis, MO, USA).

Preparation of *Aspergillus* suspensions

A. flavus and *A. parasiticus* were subcultured in Potato Dextrose Agar (PDA) (Merck Co., Germany) at 28ºC for 5 days. Then, 10 ml of PST solution (Physiological Salt Solution Containing 0.01% Tween 80) was poured on the surface of the colonies and gently scraped with a U-shaped glass rod. The resulting suspension was kept at room temperature without movement for 15 min, so that possible hyphae fragments were precipitated, and then the number of conidia present in each milliliter of the suspension was counted using a hemocytometer slide. The final concentration of the suspension was 2×10^6 conidia/ml.

Microdilution broth assay

The MIC and MFC values of berberine salts were evaluated based on the Clinical and Laboratory Standards Institute (CLSI) document M38-A2 with some modifications (CLSI, 2008). RPMI 1640 medium containing 3-(N-morpholino) propane sulfonic acid (MOPS) buffer was prepared according to CLSI standard instruction and its pH was set to 7. Finally, the medium was sterilized using a 0.22 µ syringe filter. At first, two-fold serial dilutions of berberine sulfate and berberine chloride were prepared in RPMI 1640 medium in rows of 96 cell culture plates. Each well in the row contained 100 µl of different dilutions of berberine salts ranging from 2000

to 15.6 µ/ml. Then, 100 µl of fungal suspension with a concentration of 0.4-5 $\times 10^4$ Conidia/ml was inoculated into each well and the plates were incubated for 48 h at 28°C. For each experiment, a positive control without berberine and containing fungi and a negative control without berberine and fungi were considered. All tests were performed in triplicate. The MIC was defined as the lowest concentration of completely inhibiting the growth of fungi. The MFC of berberine salts was determined by culturing from the MIC well and subsequent wells in PDA for 7 days at 28°C. Concentrations in which no fungi were grown or less than three colonies were considered as MFC (CLSI, 2008).

Effect of berberine salts on the radial growth of *A. flavus* and *A. parasiticus*

The effect of berberine salts on the radial growth was measured through culture in solid medium. Briefly, PDA plates containing 125, 250, 500, 1000, and 2000 µg/ml of berberine salts were prepared and a sterile 5 mm blank disk was placed in the center of each plate. Ten microliters of *Aspergillus* suspensions containing 2×10^6 conidia/ml was inoculated into the disks. A plate without berberine was selected as a control for each species. The plates were incubated at 28°C and the average diameter of the colonies was measured after the incubation period. The antifungal effect was calculated as the percentage of radial growth inhibition according to the following equation:

$$(\%) = \frac{D_c - D_s}{D_c} \times 100$$

D_c represents the fungal colony diameter in the control plate and D_s represents the fungal colony diameter in the treated plates.

The effect of berberine salts on aflatoxin production by *A. flavus* and *A. parasiticus*

Berberine sulfate and berberine chloride at concentrations of MIC/2 and MIC/4 were added to 50 ml of flasks containing yeast extract broth (YEB) (Merck Co., Germany), and then the flasks were inoculated with a concentration of 1.5×10^6 conidia/ml. The flasks were kept for 10 days in an incubator with a temperature of 28°C and a rotation of 100 rpm. Also, flasks containing YEB without fungal inoculation were considered as negative control and flasks containing YEB without berberine as positive control.

Aflatoxin production assay

For evaluation of aflatoxin formation, berberine sulfate and berberine chloride at concentrations of MIC/2 and MIC/4 were used. Spore suspension (1.5×10^6 conidia/ml) was added to 50 ml of flasks containing YEB containing different concentrations of berberine sulfate and berberine chloride. The flasks were kept for 10 days in an incubator with a temperature of 28°C and a rotation of 100 rpm. After the incubation period, cultures were autoclaved at 121°C

for 30s, to inactivate mycelia and conidia, and filtered through Whatman No. 1 filter paper. The mycelia were dried to a constant weight at 80°C and the weight of dried matter was estimated. Determination of aflatoxins B1, B2, G1, and G2 was performed by immunoaffinity column extraction using RP-HPLC according to AOAC. Briefly, the filtrated content of each flask was mixed with 150 ml MeOH: H2O (80:20) and 2.5 g NaCl, followed by vortexing for three min. Sixty-five microliter of phosphate buffer solution (PBS) was added to 10 ml of this mixture, shaken vigorously and passed through glass fiber filter. Seventy ml of solution was transferred onto an immunoaffinity column (Puri-Fast-AFLA IAC, Libios, France) in a flow rate of 3 ml/min. The column was then washed with 15 ml PBS, dried by passing air gently through it and aflatoxins were eluted with adding 500 and 750 µl methanol with 1 min interval. The elution diluted with 1750 µl H_2O and the aliquot of 200 µl was injected into HPLC system equipped with a separator module (2695, Waters, USA), a Nova-Pak LC-18 column and a fluorescence detector (474, Waters, USA). Aflatoxins were derivatized by KB Cell post column derivatization system (Libios, Chemin de plagne 69210 Bully, France) in a H_2O–MeCN–MeOH mobile phase containing HNO_3 and KBr at a flow rate of 1 ml/min and detected at an excitation wavelength of 365 nm and an emission wavelength of 435 nm. Quantization of aflatoxins was performed using the peak height by Millenium 32 v 4.0 software (Waters, USA). Aflatoxin standards were purchased from Sigma (St. Louis, MO, USA). The percent inhibition of aflatoxin production was calculated by the following equation:

Inhibition of aflatoxin production (%) = $\frac{A_c - A_s}{A_c} \times 100$

where *Ac* is the amount of aflatoxin in control sample, *as* is the amount of aflatoxin in treated sample (Hassan *et al*., 2015).

Statistical analysis

The quantitative data of fungal growth and HPLC analyses were subjected to variance (One-way ANOVA) in Tukey range (SPSS, version 16). The differences with $p < 0.05$ were considered significant.

Results

Minimum inhibitory concentration (MIC) and minimum lethal concentration (MFC)

As shown in **Table 1**, based on broth microdilution method, berberine sulfate showed the MIC values of 250 and 500 µg/ml for *A. flavus* and *A. parasiticus*, respectively. Berberine chloride exhibited the stronger activity than berberine sulfate, with MIC values of 125 and 250 µg/ml against *A. flavus* and *A. parasiticus*, respectively. Subcultures of these treated inoculums were negative, confirming fungicidal effects (MFC) against *A. flavus* and *A. parasiticus* at concentrations of 500 to 2000 µg/ml (**Table 1)**.

The effect of berberine sulfate and chloride on the growth of *Aspergillus parasiticus* and *Aspergillus flavus*

As demonstrated in Table 2 and Figure 1. All concentrations of berberine sulfate and berberine chloride exhibited significant inhibition of radial growth of *A. flavus* and *A. parasiticus* in comparison to control group, suggesting a dose-dependent pattern ($P< 0.05$). Berberine sulfate (2000 µg/ml) and berberine chloride (1000 µg/ml) exhibited a growth inhibition percent of mycelia production by *A. flavus* in value of 100%. In addition, berberine sulfate at concentration of 2000 µg/ml and berberine chloride at concentration of 1000 µg/ml inhibited the radial growth of mycelia production by *A. parasiticus* in values of 96.7 and 100%, respectively (**Table 2**).

The effect of berberine chloride and berberine sulfate on aflatoxin production

In our study, when the exact concentrations (MIC/2 and MIC/4) of berberine sulfate and berberine chloride were added to the cultures, significant reductions in aflatoxins production were observed by *A. flavus* and *A. parasiticus* in comparison to control ($P< 0.05$) (**Tables 3 and 4, and Figure 2**). As shown in **Table 3**, berberine chloride exhibited higher inhibitory effect on aflatoxins production than berberine sulfate by *A. flavus* ($P< 0.05$). Berberine chloride caused significant reductions in value of 100% for aflatoxine G1 and aflatoxine G2 by *A. flavus*. According to **Table 4**, aflatoxins

production by *A. parasiticus* treated with berberine sulfate and berberine chloride at MIC/2 concentration was significantly lower than MIC/4 concentration ($P< 0.05$). Also, berberine chloride exhibited higher inhibitory effect on aflatoxins production than berberine sulfate by *A. parasiticus* (**Figure 2**). Berberine chloride caused significant reduction in value of 100% for aflatoxine G1 by *A. parasiticus* (**Table 4**). At MIC/2 concentration, berberine chloride decreased aflatoxin production by *A. flavus* and *A. parasiticus* in values of 96.81% and 98.12%, respectively, 100% for aflatoxine B2, 98.9% for aflatoxine G1, 100% for aflatoxine G2 and 97.5% for total aflatoxin ($P<0.05$) (**Figure 2**).

Discussion

In the present study we showed a new biological activity for berberine as inhibitor of aflatoxins B1, B2, G1, and G2 by *A. flavus* and *A. parasiticus* in addition to its ability for strong fungal growth inhibition. MIC and MFC techniques were employed to assess fungistatic and fungicidal properties of berberine sulfate and berberine chloride. Several studies have been carried out on the chemical composition of *Berberis vulgaris* and have shown that the most important constituents of this plant are isoquinoline alkaloids such as berberine (Tabeshpour *et al*., 2017). In the case of antifungal effects of berberine, few studies approved the high potential of berberine against some pathogenic fungal strains (Da Silva *et al*., 2016; Mahmoudvand *et al*.,

2014). According to Ghareeb *et al*. (2013) study, a 62% berberine ethanolic extract from dried *Berberis vulgaris* roots displayed antifungal activity against five fungal infections at dosages ranging from 1:1–1:8 (*Penicillium verrucosum*, *Fusarium proliferatum*, *A. parasiticus*, *A. niger*, and *A. flavus*) (Ghareeb *et al*., 2013). In a study by El-Zaher (2022), the MIC values of *Berberis vulgaris* leaf and root extracts for *A. flavus* were 70 and 90 µg/ml, respectively, while these values for *A. parasiticus* were found to be 85 and 100 µg/ml (El- Zahar *et al*., 2022). Lei *et al*. (2011) showed that the MIC range of berberine over the 42 strains (*Aspergillus* spp.) was 4–256 µg/ml (Lei *et al*., 2011). The growing rate of *Trichophyton mentagrophytes* treated with berberine hydrochloride was significantly lower than those obtained in untreated control, demonstrating that berberine hydrochloride was fungicidal (Xiao *et al*., 2019). Additionally, few studies have found that *Berberis vulgaris* and its major component, berberine, have antifungal action against *Candida* spp. In a study conducted by da Silva *et al*. (2016), fluconazole resistant *Candida* and *Cryptococcus neoformans* strains showed berberine MICs equal to 8 µg/ml and 16 µg/ml, respectively (Da Silva *et al*., 2016). Cytometric analysis showed that treatment with berberine caused alterations to the integrity of the plasma and mitochondrial membranes and DNA damage, which led to cell death, probably by apoptosis (Da Silva *et al*., 2016; Li *et al*., 2013). demonstrated that berberine has a strong antifungal effect on *C. albicans*, causing cell cycle arrest and DNA damage. Other studies have also suggested that the berberine can bind to DNA, affecting DNA replication and transcription and the cell cycle (Bhadra and Kumal, 2011).

In this study, berberine salts showed an inhibitory effect on the radial growth of A. flavus and A. parasiticus mycelium (Table 2). El-Zahar *et al*. (2022) showed that *Berberis vulgaris* root extract inhibited the mycelial growth of *Penicillium verrucosum*, *Fusarium proliferatum*, *A. ochraceous*, *A. niger*, and *A. flavus*. For *P. verrucosum* and *A. ochraceous*, the maximum inhibition zones ranged from 1.7 to 2.35 cm at the 100 µl concentration (El- Zahar *et al*., 2022). In a study by Lei *et al*. (2011), *Aspergillus* treated with berberine exhibited smaller colony size, slower mycelial growth, and reduced conidia. These cultures also lost conidial pigment such that the conidial surface observed was white rather than green-gray (Lei *et al*., 2011). These results demonstrated that berberine can restrain *Aspergillus* growth, development and conidial pigmentation. Some studies demonstrated that berberine significantly inhibits gene expression in the *Aspergillus* ergosterol biosynthesis pathway and that berberine is significantly more effective than azoles at inhibiting expression of the *Erg5*, *Cyp51A*, *Cyp51B* and *IMP* genes, which are related to pigment production in *Aspergillus* conidia. The *IMP* gene is closely related to cell wall biosynthesis and, by inhibiting its expression, berberine may thus inhibit biosynthesis of fungal cell walls and cause growth and developmental aberrations in *Aspergillus* (Ouyang *et al*., 2010). da Silva *et al*. (2016) demonstrated that the berberine concentration necessary to inhibit both planktonic cells and preformed biofilm cells is similar (Da Silva *et al*., 2016). This finding indicated that berberine may reduce the growth of planktonic cells and inhibit the viability of cells in preformed biofilms at concentrations of 8 µg/ml and 37.5 µg/ml, respectively.

Up to now, there has been no research on aflatoxins inhibition by berberine, but a few investigations reported the effect of *Berberis vulgaris* on aflatoxin production. In this regard, Ghareeb *et al*. (2013) showed that ethanolic extract of *B. vulgaris* was able to inhibit the production of 44% and 98.3% of aflatoxine B1 and 67.2 and 89% of aflatoxine B2 at concentrations of 0.01 to 0.1%, respectively (Ghareeb *et al*., 2013). Safari *et al*. (2020) exhibited that the inhibition of aflatoxin B1 production by *A. flavus* in *B. vulgaris* extract (6 mg/ml) was significant (Safari *et al*., 2020). Their findings demonstrated a highly significant correlation between the gene expression and the aflatoxin B1 biosynthesis, such that certain doses of the extract reduced or blocked the expression of the *aflR*, *aflM* and *aflP* and consequently reduced the synthesis of aflatoxin B1. Interestingly, compared to the regulatory gene (*aflR*), the down-regulation of expression in the structural genes (*aflM* and *aflP*) was more consistent and correlated with the inhibition of aflatoxin B1 production. In another study by Tintu *et al*. (2012), the alpha amylase inhibitors, such as berberine, can be used to control the growth of *A. flavus* as well as the production of aflatoxins (Tintu *et al*., 2012). Malekivezhad *et al*. (2021) showed that addition of different levels of berberine to chickens challenged with aflatoxin reduced the negative effect of this toxin on broiler feed intake (Malekivezhad *et al*., 2021). Also, supplementation of aflatoxin B1-contaminated diets with berberine improved growth performance and reduced vascular congestion, inflammatory cell infiltration into the liver portal space, and hepatocyte apoptosis. Furthermore, it protected against toxin induced damage to the

ileal epithelium. These findings suggested that berberine could be a useful dietary strategy to prevent effects of aflatoxicosis in animals and human.

Conclusions

In summary, our findings indicated the potential of berberine as a natural inhibitor of the growth and aflatoxins production by *A. flavus* and *A. parasiticus*, the well-known causal agents of food-borne aflatoxicosis.

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اثر بربرین سولفات و بربرین کلرید در شرایط برون تنی بر رشد و تولید آفلاتوکسین توسط *آسپرژیلوس فلاووس* و *آسپرژیلوس پارازیتیکوس*.

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چکیده:

زمینه مطالعه: آفلاتوکسین ها، سموم قارچی مضری هستند که می توانند خوراک دام و محصولات غذایی را آلوده کنند. ترکیبات گیاهی به عنوان عوامل بالقوه برای مهار رشد و تولید آفلاتوکسین توسط قارچهای توکسین زا مورد بررسی قرار گرفته‌اند

هدف: این مطالعه با هدف بررسی اثر بربرین سولفات و بربرین کلرید در شرایط آزمایشگاهی بر رشد و تولید آفلاتوکسین در *آسپرژیلوس فلاووس* و *آ. پارازیتیکوس* انجام شد

مواد و روش کار: فعالیت ضد قارچی نمک های بربرین بر اساس سند M38-A3 موسسه استانداردهای بالینی و آزمایشگاهی (CLSI)تعیین گردید. سطح آفلاتوکسین با استفاده از روش کروماتوگرافی مایع با کارایی بالا (HPLC) اندازه گیری شد.

نتایج: حداقل غلظت بازدارندگی بربرین سولفات و بربرین کلرید علیه *آسپرژیلوس فلاووس* به ترتیب ۲۵۰ و ۱۲۵ میکروگرم بر میلی لیتر بود. این مقادیر برای *آسپرژیلوس پارازیتیکوس* به ترتیب ۵۰۰ و ۲۵۰ میکروگرم بر میلی لیتر محاسبه شد. بربرین سولفات با غلظت ۲۰۰۰ میکروگرم بر میلی لیتر و بربرین کلرید با غلظت ۱۰۰۰ میکروگرم در میلی لیتر منجر به مهار کامل رشد میسلیوم *آسپرژیلوس فلاووس* شد. علاوه بر این، بربرین سولفات با غلظت ۲۰۰۰ میکروگرم در میلی لیتر باعث کاهش ۹۶,۷ درصدی رشد میسلیوم *آسپرژیلوس پارازیتیکوس* شد، در حالی که کلرید بربرین با غلظت ۱۰۰۰ میکروگرم در لیتر منجر به مهار ۱۰۰ درصدی رشد میسلیوم شد.

نتیجه گیری نهایی: نمک های بربرین تولید آفلاتوکسین کل توسط هر دو گونه *آسپرژیلوس* را در غلظت های MIC/2 و MIC/4به طور معنی داری کاهش دادند .($P < 0/05$) نتایج نشان می‌دهد که نمک‌های بربرین می‌توانند به‌عنوان عوامل ضد قارچی و ضد آفلاتوکسیژنیک بالقوه در برابر جدایه‌های سمی *آسپرژیلوس* استفاده شوند.

کلمات کلیدی: آفلاتوکسین ها، *آسپرژیلوس فلاووس*، *آسپرژیلوس پارازیتیکوس*، بربرین، رشد میسلیوم.

455 **Table 1.** Anti-*Aspergillus* susceptibility of berberine sulfate and berberine chloride based on microdilution broth method.

Test Compound	MIC (µg/ml)		MFC (µg/ml)	
	Aspergillus flavus	*Aspergillus parasiticus*	*Aspergillus flavus*	*Aspergillus parasiticus*
Berberine sulfate	250	500	1000	2000
Berberine chloride	125	250	500	1000

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Table 2. The effects of berberine sulfate and berberine chloride on the radial growth of *Aspergillus flavus* and *Aspergillus parasiticus*.

Berberine concentration (µg/ml)	*Aspergillus flavus*				*Aspergillus parasiticus*			
	Berberine sulfate		**Berberine chloride**		**Berberine sulfate**		**Berberine chloride**	
	Colony diameter (mm)	Growth inhibition (%)	Colony diameter (mm)	Growth inhibition (%)	Colony diameter (mm)	Growth inhibition (%)	Colony diameter (mm)	Growth inhibition (%)
0	41±1	0	41±1	0	36.8±1.4	0	36.8±1.4	0
125	29.2±0.8	28.8	18.8±0.84	54.1	29.4±0.5	20.1	22.2±1.8	39.7
250	14±1.2	65.9	10.8±1.1	73.7	24.6±0.9	33.2	16.8±1.1	54.3

500	10±2	75.6	1.2±1.1	97.1	16±1.4	56.5	6±1.4	83.7
1000	1.6±1.7	96.1	0	100	7.2±1.8	80.4	0	100
2000	0	100	0	100	1.2±1.8	96.7	0	100

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Table 3. The mean ± standard deviation (SD) of aflatoxin concentration by *Aspergillus flavus* treated with berberine sulfate and chloride.

Test	Concentration	Aflatoxin (µg/l)				
		B1	**B2**	**G1**	**G2**	**Total**
Control		538.38±2.58	41.13±0.49	211.98±4.37	2.86±0.08	794.34±2.2
berberine sulfate	**MIC/2**	173.95±4.17	19.75.2±1.2	41.63±1.67	1.19±0.07	236.52±3.63
	MIC/4	436.37±2.61	34.39±1.86	98.51±1.26	2.1±0.22	571.38±2.9
berberine chloride	**MIC/2**	24.48±0.63	0.8±0.8	0	0	25.28±1.43
	MIC/4	170.05±6.51	15.7±1.06	16.8±0.99	0	202.55±8.56

Table 4. The mean ± standard deviation (SD) of aflatoxin concentration by *Aspergillus parasiticus* treated with berberine sulfate and chloride.

Test	Concentration	Aflatoxin (µg/l)				
		B1	B2	G1	G2	Total
Control		1642.25±9.50	112.74±4.72	980.68±7.07	25.53±3.96	2761.20±17.60
berberine sulfate	MIC/2	542.33±4.84	28.07.2±2.37	348.79±4.26	79.7±1.20	926.98±12.67
	MIC/4	909.21±12.09	39.55±2.97	748.26±12.27	12.27±0.88	1709.28±65.16
berberine chloride	MIC/2	24.48±0.63	0.8±0.8	0	10.5±0.42	51.65±15.3
	MIC/4	170.05±6.51	15.7±1.06	291.36±29.5	18.08±0.17	1151.86±18.5

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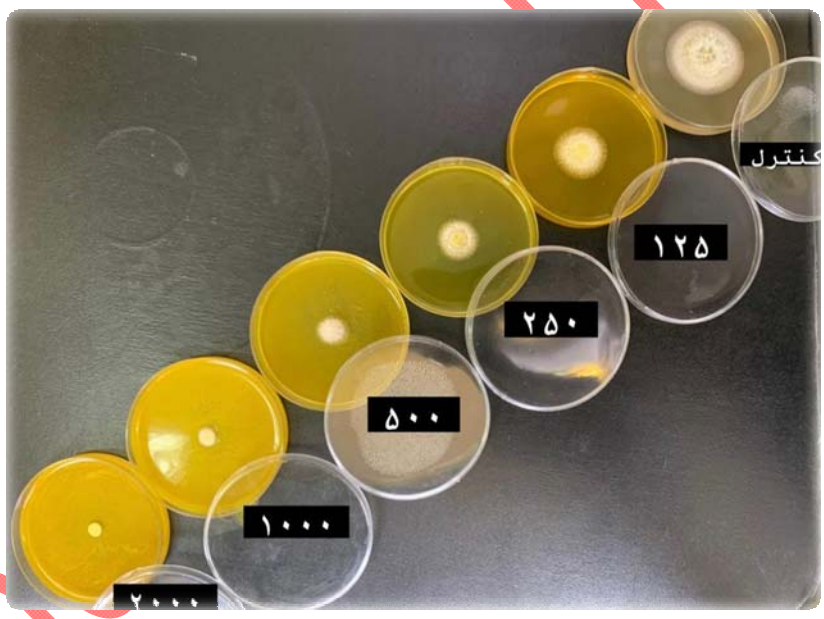

Figure 1. The effect of berberine chloride on the growth of *Aspergillus flavus* colonies after 7 days

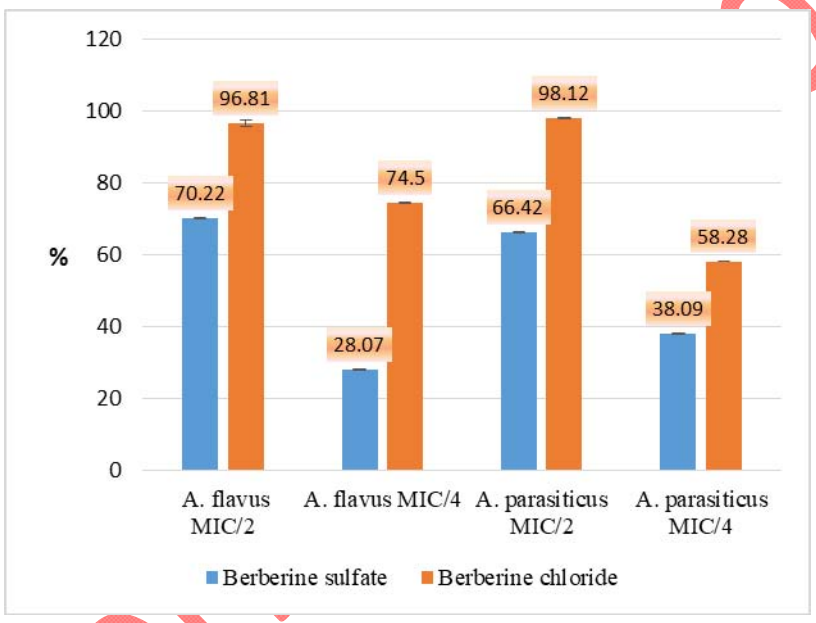

Figure 2. Comparison of the effect of different concentrations of berberine sulfate and berberine chloride on total aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*.