Biological activities of *Boswellia sacra* extracts on the growth and aflatoxins secretion of two aflatoxigenic species of *Aspergillus* species

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**Abstract**

Aflatoxins are the most serious carcinogenic, hepatotoxic, teratogenic and mutagenic secondary metabolites which adversely affect human and animal health. This study was designed to evaluate the in vitro inhibitory effect of different concentrations of *Boswellia sacra* resin (2.5, 5, 7.5 and 10 g/100 ml), leaf extract (5, 7.5, 10, 12.5 and 15 ml/100 ml), and essential oil (1, 2, 3, and 4 ml/100 ml) on the growth and aflatoxins production by two species of *Aspergillus*, namely *Aspergillus flavus* (SQU21) and *Aspergillus parasiticus* (CBS9217). Resin of *B. sacra* caused 57.9–92.1% inhibition of aflatoxin secretion by *A. flavus* and 43.6–95.7% for *A. parasiticus*. However, the mycelial dry weights were significantly increased by 20.9–52.7% for *A. flavus*, and 8.9–68.5% for *A. parasiticus*. The leaf extract of *B. sacra* apparently enhanced aflatoxins production by 20–50%, and mycelial dry weight by 25.5–29.1% for *A. flavus* and *A. parasiticus*. The essential oil of *B. sacra* at different concentrations similarly inhibited the fungal growth and aflatoxins production by 45.8–83.7% for *A. flavus* and 41.3–83.5% for *A. parasiticus* which indicates the antifungal activity of this oil. None of the *B. sacra* extracts detoxified pure aqueous aflatoxin B1. We have concluded that *B. sacra* resin and essential oil possess biological activity against biochemical synthesis and metabolic pathway of aflatoxin production of the two *Aspergillus* species. Therefore, the resin and essential oil of *B. sacra* can be recommended as safe plant based bioremediative to enhance shelf life of food and feed products with reference to adverse effect of physical and synthetic chemical preservatives and their antimicrobial and aflatoxins inhibition activity.

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1. Introduction

The genus *Boswellia* of the family Burseraceae includes eight species that grow in the Arabian Peninsula, India and East Africa (Gamarda, Dayton, Distefano, Pitonzo, & Schillaci, 2007; Hasson et al., 2011; Miller & Morris, 2004). There are many varieties of *Boswellia serrata* in India, *Boswellia carteri* in East Africa and China, *Boswellia frereana* in Somalia, and *Boswellia sacra* in Oman, Island of Soqotra (Yemen). They produce slightly different types of resin known as frankincense (Olibanum, oleogum), “Luban” in Arabic and “Levohah” in Hebrew (Hasson et al., 2011). Frankincense resin has been important for civilizations of the Arabian Peninsula and North Africa as precious commercial product and incense material (Mothana, Hasson, Schultz, Mowitz, & Lindequist, 2011). The chemical nature of *Boswellia* species has been reported by many researchers. These investigations led to identification of 67–72 chemical constituents in different *Boswellia* species such as essential oil with monoterpen hydrocarbon (32.8%), α-thujene (9.3%), α-pine (8.3%), diterpene (31.7%), incensol (14.8%), and oxygenated monoterpenes (30.7%) with *p*-cymene (13%), 2-hydroxy-5-methoxy-acetophenene (16.3%), and camphore (11.6%) (Al-Harrasi & Al-Saidi, 2008; Mothana et al., 2011). In addition, frankincense has been reported to be a rich source of non-volatile triterpenic constituents from the types of ursane, oleane, and lupine, which are in many cases responsible for various biological activities (Büchele, Zugmaier, & Simmet, 2003; Safayhi & Sailer, 1997; Singh et al., 2008). *Boswellia* species is among the most important medicinal plants, which are used for relieving fever and pains as well as disturbed stomach (Miller & Morris, 2004; Mothana et al., 2011). Traditionally, the frankincense resin has been used for the treatment of rheumatic and other inflammatory diseases (Banno et al.,

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2006; Langmead & Rampton, 2006; Mothana et al., 2011). Toxicity studies show that resin preparations could be effective in the treatment of chronic colitis with minimal side effects (Gupta et al., 2001). Supplementation of different plant extracts including resin from Boswellia was beneficial in modulating the alteration induced in kidney and heart of animals under toxic effect of AFB1 (Abulmajeed, 2011). Essential oil prepared from hydrodistillation has tumor cell-specific cytotoxicity in multiple cancer cell types (Suhail et al., 2011). Therefore, it has gained obvious attention of researchers and pharmaceutical companies. Several studies have reported on the antitumor, anti-inflammatory, immunomodulatory, and antiviral activities of several Boswellia species (Akhhsa et al., 2006; Ammon, Safayhi, Mack, & Sabieraj, 1993; Badria, Mkhkaei, Maatooq, & Amer, 2003; Banoo et al., 2006; Mkhkaei, Moatooq, Badria, & Amer, 2003; Mothana & Lindequist, 2005; Mothana, Mentel, Reiss, & Lindequist, 2006; Mothana et al., 2011). This prompted us to evaluate the antifungal properties and detoxification activities of these extracts on aflatoxigenic Aspergillus species.

Moulds are significant decomposers that adversely affecting human food and animal feed, by retarding their nutritive value and producing toxigenic secondary metabolites (El-Nagerabi, Al-Bahry, Elshafee, El-Nagarabi, Al-Bahry, & Elamin, 2013; Hajjah, 2009; Jayaprakasha, Selvi, Jena, & Sakariah, 2005; Peraica, Domijan, Ozan, & Cvetkovic, 2002; Wild & Turner, 2002). The vegetative growth and associated aflatoxins produced by A. flavus, and A. parasiticus have been proven to be sensitive to different plant extracts and essential oils (EOs) extracted from various medicinal and herbal plants (El-Nagerabi, Al-Bahry, et al., 2012; Elshafee, El-Mubarak, El-Nagerabi, & Elshafee, 2010). Aflatoxins in general (AFB1, AFB2, AFG1, and AFG2) and particularly Aflatoxin B1 are the most serious threat to public health due to their carcinogenic, hepatotoxic, and mutagenic effects for both human and domestic animals (Abdulmajeed, 2011). Several studies revealed the antimicrobial and antiviral properties of different species of Boswellia plant (Mothana & Lindequist, 2005; Mothana et al., 2006, 2011). Therefore, the present study was designed to evaluate the in vitro biological effects of frankincense resin, green leaf extract, and essential oil of B. sacra from Oman on the mycelial growth and aflatoxins production by two Aspergillus species, namely A. flavus (Strain SQU21) and A. parasiticus (Strain CBS921.7). We anticipate these findings will lead to major development in food production and preservation technology.

2. Materials and methods

2.1. A. flavus and A. parasiticus isolates

Two species of high aflatoxin-producers namely A. flavus (SQU21) and A. parasiticus (CBS921.7) [NRRL22999] were obtained from the culture collection of Sultan Qaboos University, Oman. The isolates were inoculated on Potato Dextrose Agar (PDA) and identified according to Raper and Fennell (1965).

2.2. Sources and characteristics of B. sacra extracts

In this study, frankincense resin powder, green leaf, and oil were used. The resin is obtained through incisions made in the trunks of Boswellia trees and the resin oozes from the incisions, allowed to dry for 3–4 days, manually collected, cleaned from plant debris, and sent to the market without any other treatment. The resin granules were
were selected and added separately to different 100 ml sterile distilled water. The highest concentrations of resin were prepared and the mycelia of the fungi were incubated for 10 days. The concentration of the aflatoxin (0.0%, 5%, 7.5%, 10%, 12.5% and 15% v/v), or oil (0.0%, 1%, 2%, 3% and 4% v/v) of frankincense extract (0.0%, 5 g, 7.5 g, 10 g) was set. The leaf extract was prepared by mixing 50 g of leaves with 200 ml distilled water and grinding this mixture using an automatic blender at high speed for 2 min. The mixture was filtered through Whatman filter paper No. 4. The filtrate was kept in a refrigerator at 4°C for 15 days. Another sets were prefiltered and the dry weight of the tested species were significantly (p < 0.05) increased with concentrations of resin (2.5, 5, 7.5, and 10 g/100 ml) in comparison with the control. The percentages of aflatoxins inhibition ranged between 57.9–92.1% and 43.6–95.7% for *A. flavus* (SQU21) and *A. parasiticus* (CBS921.7), respectively (Fig. 1). On the contrary, the mycelial dry weights of the tested species were significantly (p < 0.05) increased with concentrations of resin (Fig. 2). The dry weight was enhanced by 20.9–52.7% for *A. flavus* (SQU21) and 8.9–68.5% for *A. parasiticus* (CBS921.7).

It is commonly known that not all isolates of *Aspergillus* are able to produce aflatoxin; however, more than 50% of these isolates are aflatoxin-producers secreting different types of aflatoxins (El-Nagerabi, Al-Bahry, et al., 2012; El-Nagerabi, Elshafie, et al., 2013; Lisker, Michaeli, & Frank, 1993). The two species used in the present study produce high concentrations of aflatoxins and were recently used in similar investigations (El-Nagerabi, Al-Bahry, et al., 2012; El-Nagerabi, Elshafie, et al., 2013). Numerous studies have been conducted on anticancer, anti-inflammatory, immunomodulatory, antimicrobial, and antiviral activities of many species of *Boswellia* (Akihisa et al., 2006; Ammon et al., 1993; Banno et al., 2006; Badria et al., 2003; Mikhail et al., 2003; Mothana & Lindequist, 2005; Mothana et al., 2007; Montes-Belmont & Carvajal, 1998; Patkar et al., 1993; Soher, 1999; Soliman & Badea, 2002). In the present study, the effects of different concentrations of frankincense resin, green leaf extract, and essential oil of *B. sacra* on the growth and aflatoxins production of *A. flavus* (SQU21) and *A. parasiticus* (CBS921.7) were investigated. The results showed that the total aflatoxins produced by the two species were significantly (p < 0.05) inhibited by all tested concentrations of frankincense resin (2.5, 5, 7.5, and 10 g/100 ml) in comparison with the control.

### 2.3. Inoculation of *Aspergillus* species on media containing frankincense extracts

*A. flavus* (SQU21) and *A. parasiticus* (CBS921.7) were inoculated on Potato Dextrose Agar (PDA) and incubated at ambient temperature (25–29°C) for 10 days. After the incubation period, sterile thin glass tubes of 5 mm in diameter were used to cut several small circular discs from the sporulating cultures. Two discs were added aseptically to 200 ml of sterile yeast malt broth in 250 ml conical flasks containing resin (0.0%, 2.5%, 5.0%, 7.5% and 10.0% w/v), leaf extract (0.0%, 5%, 7.5%, 10%, 12.5% and 15% w/v), or oil (0.0%, 1%, 2%, 3% and 4% v/v) of frankincense (olibanum). As a negative control, 10% resin, 15% leaf extract and 4% oil were added to yeast malt broth without any fungal inoculation. Triplicates of the inoculated flasks were incubated at 25–29°C for 15 days. Another sets were prepared and the mycelia of the fungi were filtered and the dry weight was determined using the Oven method.

### 2.4. Effect of frankincense extracts on pure aflatoxin B₁

Pure aflatoxin B₁ of 870 ppb concentration was prepared in 100 ml sterile distilled water. The highest concentrations of resin (10 g/100 ml) and leaf extract (15 ml/100 ml) and oil (4 ml/100 ml) were selected and added separately to different flasks containing pure aflatoxin B₁. As a control, flask containing aflatoxin B₁ and without any extract was set. The flasks were incubated at 25–29°C for 10 days. The concentration of the aflatoxin B₁ was measured.

### 2.5. Extraction and determination of aflatoxin by Alfa Test-P Affinity

For aflatoxin extraction, the 200 ml fungal culture were mixed with 5 g of NaCl salt and 100 ml extraction solution of methanol:water (70:30 V/V) as described by El-Nagerabi, Al-Bahry, et al. (2012). The content was then blended at high speed for 1 min and filtered; 15 ml of the filtrate were diluted with 30 ml distilled water, mixed thoroughly and filtered through glass microfilters. Ten milliliter from the diluted filtrate (10 ml = 1.0 g sample equivalent) were passed through Alfa-Test-P Affinity Column at a rate of 1–2 drops per second. The column was then cleaned using 10 ml distilled water, and the aflatoxin was eluted with one ml methanol (HPLC grade). Then, 1 ml of Alfa-Test developer was added to the elute in the cuvette, and mixed. The blank contains 1 ml methanol and aflatoxin developer. The concentration of the aflatoxin was measured using calibrated and adjusted Vicam fluorometer (Series-4EX) with excitation wavelength of 360 nm and emission wavelength of 440 nm (Elshafie & Al-Shally, 1998).

### 2.6. Statistical analysis

A one-way ANOVA test (correlation coefficient) was used to determine the variation between the effect of different concentrations of *B. sacra* extracts on aflatoxin secretion and mycelial dry weight compared to the control. The analysis was carried out using SPSS software (version 11.0).

### 3. Results and discussion

The potential effects of certain plant extracts and biocontrol agents on the fungal growth and aflatoxins production have been under investigation by many authors (Gowda et al., 2004; Joseph et al., 2005; Reddy, Reddy, & Muralidharan, 2009; Suleiman, Emuwa, & Taiga, 2008). Much emphasis was given to herbal, medicinal and aromatic plants for their antifungal activities against food spoilage and aflatoxigenic fungi (El-Nagerabi, Al-Bahry, et al., 2012; El-Nagerabi, Elshafie, et al., 2013; Gandomi et al., 2009; Maraqa et al., 2007; Montes-Belmont & Carvajal, 1998; Patkar et al., 1993; Soher, 1999; Soliman & Badea, 2002). In the present study, the effects of different concentrations of frankincense resin, green leaf extract, and essential oil of *B. sacra* on the growth and aflatoxins production of *A. flavus* (SQU21) and *A. parasiticus* (CBS921.7) were investigated. The results showed that the total aflatoxins produced by the two species were significantly (p < 0.05) inhibited by all tested concentrations of frankincense resin (2.5, 5, 7.5, and 10 g/100 ml) in comparison with the control. The percentages of aflatoxin inhibition ranged between 57.9–92.1% and 43.6–95.7% for *A. flavus* (SQU21) and *A. parasiticus* (CBS921.7), respectively (Fig. 1). On the contrary, the mycelial dry weights of the tested species were significantly (p < 0.05) increased with concentrations of resin (Fig. 2). The dry weight was enhanced by 20.9–52.7% for *A. flavus* (SQU21) and 8.9–68.5% for *A. parasiticus* (CBS921.7).

Fig. 1. Aflatoxin production of *A. flavus* strain SQU21 and *A. parasiticus* strain CBS921.7 at different concentrations of *B. sacra* resin (identical letters and numbers indicate no significant difference, p > 0.05).

It is commonly known that not all isolates of *Aspergillus* are able to produce aflatoxin; however, more than 50% of these isolates are aflatoxin-producers secreting different types of aflatoxins (El-Nagerabi, Al-Bahry, et al., 2012; El-Nagerabi, Elshafie, et al., 2013; Lisker, Michaeli, & Frank, 1993). The two species used in the present study produce high concentrations of aflatoxins and were recently used in similar investigations (El-Nagerabi, Al-Bahry, et al., 2012; El-Nagerabi, Elshafie, et al., 2013). Numerous studies have been conducted on anticancer, anti-inflammatory, immunomodulatory, antimicrobial, and antiviral activities of many species of *Boswellia* (Akihisa et al., 2006; Ammon et al., 1993; Banno et al., 2006; Badria et al., 2003; Mikhail et al., 2003; Mothana & Lindequist, 2005; Mothana et al., 2006, 2011). To our knowledge, the detoxification properties of resin, leaf and oil extract of *B. sacra* on aflatoxigenic fungi had not been evaluated. This enforces the need for investigating the inhibitory effects and detoxification properties of *B. sacra* extracts and further identification of the biologically active chemical ingredients. In the present investigations, the concentrations of frankincense resin of between 2.5 and 10% resulted in 57.9–92.1%
inhibition of aflatoxins production by *A. flavus* (SQU21) and 43.6—95.7% for *A. parasiticus* (CBS921.7). In a similar study, although *Boswellia* extracts were effective against different bacterial species (Weckesser et al., 2007), neither water nor methanolic extracts revealed any activity against *C. albicans* and *C. marosoi* yeasts (Hasson et al., 2011). Other studies using different plant extracts showed apparent inhibition of the fungal growth and aflatoxin production. Plant extracts of *S. aromaticum*, *C. longa*, *A. sativum* and *O. sanctum* (5 g/kg) effectively inhibited the growth of *A. flavus* (65—78%) and AFPB1 production (72.2—85.7%) (Reddy et al., 2009). Extracts from fruit rinds of *C. cowa* and *G. pendunculata* at 2000—4000 ppb concentration completely inhibited the growth of *A. flavus* and aflatoxin B1 production up to 100% (Joseph et al., 2005). Fruit extract of *A. digitata* (baobab) (1.5, 3, 5, and 7%) apparently inhibited the total aflatoxin secretion up to 20.4—68.5% for *A. flavus* and 11.9—69.1% for *A. parasiticus*, whereas the inhibition of aflatoxin B1 production ranged from 29.9—79.2% and 13—68% for the two strains, respectively (El-Nagerabi, Elsha et al., 2013). Bullerman et al. (1977) stated that 0.02—20% cinnamon extracts inhibited aflatoxin production by 25—100%, and 2% of cinnamon led to 97% inhibition of aflatoxin secretion. The leaves and calyx extracts of *H. sabdariffa* (5—12.5%) caused 91.5—97.9% reduction in aflatoxin B1 production by *A. flavus* and *A. parasiticus* (Al-Shayeb & Mabrook, 2006). Reddy et al. (2009) found that the highest inhibition (92.1—95.7%) was obtained at 10% frankincense resin. These findings suggest the possibility of the presence of different aflatoxin inhibitors in *B. sacra* extracts which interfere with the biochemical synthesis pathways of aflatoxin. These chemicals in many cases are responsible for various biological activities of frankincense (Büchele et al., 2003; Safayhi & Sailer, 1997; Singh et al., 2008). In the present study, inoculation of the two species of *A. flavus* (SQU21) and *A. parasiticus* (CBS921.7) in yeast malt broth supplemented with different concentrations of frankincense leaf extracts (5, 7.5, 10, 12.5, and 15% v/v) significantly (p < 0.05) increased both aflatoxin production (Fig. 3) as well as the mycelial dry weights of the two *Aspergillus* species (Fig. 4). The highest concentration of the leaf extracts caused 20—50% increase in aflatoxin production, and 25.3—29.1% mycelial dry weight for *A. flavus* and *A. parasiticus*. This enhancement of aflatoxin production and the fungal vigour may be due to the high nutrient contents of the green leaves. Although, it is evident that the frankincense resin of *B. sacra* contains aflatoxin inhibitors, the leaf extracts enhanced the mycelial growth. This indicates the interference of these inhibitors with the biochemical events of aflatoxin biosynthesis as concluded by many researchers (Da Costa et al., 2010; El-Nagerabi, Al-Bahry et al., 2012; El-Nagerabi, Elshafie et al., 2013). The inhibition of the vegetative growth and aflatoxin production of *A. flavus* and *A. parasiticus* by essential oils (Eos) extracted from herbal, medicinal and aromatic plants have been recommended by many authors (ex. El-Nagerabi, Al-Bahry et al., 2012; El-Nagerabi,
Elshafie, et al., 2013; Maraqa et al., 2007; Soliman & Badeea, 2002). Many of these oils had different fungistic activities and mode of action (Gandomi et al., 2009; Shukla et al., 2012; Szczerbanik et al., 2007). They have variable effects on mycelial growth and aflatoxin production of A. flavus and A. parasiticus and associated aflatoxin secretions. It was reported that 3% of Nigella sativa oil completely inhibited aflatoxins B1, B2, G1, and G2 when using TLC for aflatoxin detection and 50% inhibition when using sensitive monoclonal antibody method (Maraqa et al., 2007). At concentration of 1–3%, this oil caused 47.9–58.3% inhibition of aflatoxin B1 for A. flavus and 32–48% for A. parasiticus (El-Nagerabi, Al-Bahry, et al., 2012). The highest inhibition levels of total aflatoxin and aflatoxin B1 secretion by A. flavus (47.2–95.7%; 28.1–89.7%) and A. parasiticus (42.7–93.3%; 25.9–80.2%) were obtained with essential oil extracted from A. digitata seeds (El-Nagerabi, Elshaie, et al., 2013). Clove oil at 200–250 ppm inhibits the growth of A. parasiticus (Pullerman et al., 1977). Essential oil of Cymbopogon flexuosus absolutely inhibits the growth of A. flavus and aflatoxin B1 production at 1.3 μM L−1 and 1.0 μM L−1, respectively. Aflatoxin B1 production by NKO-208 isolates of A. flavus was strongly inhibited at lower fungistic concentrations of essential oil of Callistemon lanceolatus (Shukla et al., 2012). The EOs of Thymus ericoides and Thymus x-porlock at 1/2–1/16 dilutions were fungicidal and strongly inhibited aflatoxin production (Rasooli & Abyaneh, 2004). Citrus exudates exhibit fungicidal activity at 1000 ppm (Mishra & Dubey, 1994). Franklinense of B. carteri at 2% (v/v) showed the strongest mycelium inhibition against A. flavus (11.1%), Fusarium moniliforme (61.1%), Fusarium proliferatum (16.7%), Pyricularia grisea (33.3%), Bipolaris oryzae (33.3%), Rhizoctonia solani (44.4%), and Alternaria brassicicola (71.3%) (Udomsip et al., 2009). In the present investigations, the effects of different concentrations of oil extracted from resin of B. sacra (1, 2, 3, and 4 ml/100 ml) on aflatoxin production (Fig. 5) and the mycelial growth rates (Fig. 6) of A. flavus (SQU21) and A. parasiticus (CBS921.7) were recorded. The results showed that the oil of B. sacra significantly (p < 0.05) reduced aflatoxin secretion for A. flavus (45.8–83.7%) and A. parasiticus (41.3–83.5%). Similarly, the mycelial dry weights of the two Aspergillus species were significantly (p < 0.05) inhibited by the tested concentrations of B. sacra oil compared to the control. This indicates the antifungal activity of B. sacra essential oil against the growth of the two species of A. flavus (SQU21) and A. parasiticus (CBS921.7). Similar conclusions were drawn by many authors using different essential oils extracted from various plants such as N. sativa and A. digitata (El-Nagerabi, Al-Bahry, et al., 2012; El-Nagerabi, Elshaie, et al., 2013; Maraqa et al., 2007).

Detoxification of aflatoxins by plant extracts is safer and more eco-friendly than detoxification by physical, chemical or microbiological means (Alberts et al., 2009; Kumar et al., 2009). The biological detoxifications offer promising alternatives for aflatoxin elimination and maintaining the quality and safety of food and feed (Alberts et al., 2009; Oguz, 2011; Prakash et al., 2011). The potential of some herbal, medicinal and aromatic plants to degrade aflatoxins has been reported by many researchers (El-Nagerabi, Al-Bahry, et al., 2012; El-Nagerabi, Elshaie, et al., 2013; Sandosskumar et al., 2007). Garlic (Allium sativum L.) and onion (Allium cepa L.) roots incubated in water containing 70 mg of aflatoxin B1 for 5 days degrade aflatoxin and cause 58.5% reduction in aflatoxin level (Velazhahan et al., 2010). The dialyzed seed extract of Trachyspermum ammi was reported to degrade 90% of aflatoxin B1 by modification of its lactone ring structure (Velazhahan et al., 2010). The aqueous extract of T. ammi seeds was reported to cause approximately 80% reduction in aflatoxin content due to the presence of aflatoxin inactivation factors (Hajare, Haijare, & Sharma, 2005). In the present study, the species of the two selected Aspergillus species are aflatoxin-producers and secreting different profiles of aflatoxins. To our knowledge, the detoxification properties of B. sacra resin, leaf and oil extracts had not been examined before. Nonetheless, the antimicrobial property of B. serrata resin was investigated. It ameliorates the deviation induced in both kidneys and hearts of animal in response to AFB1 administration (Abdulmajeed, 2011). All essential oils of Boswellia species revealed antibacterial activity against Gram-positive bacteria (Motha et al., 2011). Oil concentration of 2% evidently inhibits mycelial growth and sporulation (Udomsip et al., 2009). Therefore, it is not astonishing that the resin and oil extracts of B. sacra display enormous inhibitory effects on aflatoxin production by these aflatoxinogenic fungi. Thus, it is very important to evaluate the detoxification properties of resin and oil extracted from B. sacra to aflatoxin production and further isolation and identification of the active chemical compounds which are associated with detoxification phenomenon. In the present study, we investigated the effect of 10% (w/v) resin and 4% (v/v) oil extract of B. sacra on 870 ppb pure aqueous aflatoxin B1. The results showed that the two extracts slightly reduced aflatoxin B1 concentrations to 860 ppb and to 867 ppb in comparison with the control (870 ppb) which suggests the lack of the detoxification properties of these extracts on aflatoxin B1. Therefore, it is obvious that both resin and oil extracts had inhibitory effects on aflatoxin secretion, whereas oil possesses antifungal activity. On the other hand, resin enhanced the fungal growth of the selected Aspergillus species (A. flavus SQU21 and A. parasiticus CBS921.7). Nonetheless, none of the tested extracts (resin and oil) showed detoxification or inactivation activities against pure aflatoxin B1. However, they inhibit the biological synthesis of aflatoxin by A. flavus (SQU21) and A. parasiticus (CBS921.7), besides, the antifungal activity of oil extract.
4. Conclusion

This research investigates the biological activities of resin, essential oil, and leaf extract of B. sacra on the growth and aflatoxin secretion of A. flavus (SQU21) and A. parasiticus (CBS921.7). Our findings proved that the resin powder and essential oil of B. sacra evidently reduce aflatoxin production, whereas resin enhanced the fungal growth which indicates the inhibitory effect to aflatoxin biosynthesis and secretion pathway. The green leaf extract increases both the fungal growth and aflatoxin secretion by the two Aspergillus species. None of the extracts detoxify pure aflatoxin B1. Therefore, resin powder and essential oil can be used as natural plant additives to food and feed products based on their toxicity studies (Abulmajeed, 2011; Banno et al., 2006; Gupta et al., 2001; Langmead & Rampton, 2006; Miller & Morris, 2004; Mothana et al., 2011; Suhail et al., 2011). However, safety and toxicity of biologically active chemical ingredients which affect the fungal growth and aflatoxin production need further investigations. The identified chemical ingredients should be tested for their antifungal activity and interference with the biosynthesis pathway of aflatoxins. This will broaden our knowledge on their uses and future development in food industry and pharmaceutical applications.

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