

An *in Vitro* Antifungal and Antiaflatoxigenic Properties of *Commiphora myrrha* and *Prunus mahaleb*

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Abstract

Aflatoxins and especially aflatoxin B, are the devastating contaminant of food and feed products with hazardous effects to mankind and his domestic animals. These investigations were set to evaluate the effect of various levels of *Commiphora myrrha* resin (1.0, 1.25, 2.25, and 3.25 g/100 ml) and *Prunus mahaleb* seed extract (0.75, 1.5, 2.5, and 3.5 g/100 ml) on the growth and aflatoxin secretion by two aflatoxigenic strains of *Aspergillus flavus* and *A. parasiticus*. The two plant extracts significantly ($p < 0.05$) decreased aflatoxin secretion, and inhibited the fungal growth. Resin of *C. myrrha* displayed 51.9-95.7% reduction in total aflatoxin secretion by *A. flavus*, and 46.9-92% for *A. parasiticus*, and Seed extract of *P. mahaleb* decreased aflatoxin up to 53.7-95.8% and 40-94.7%, respectively. The inhibition of aflatoxin B (B_1 and B_2) by myrrh resin and seed extract of mahaleb ranged between 51.7-93.5, 50-93.6% (*A. flavus*) and 39.5-89.7%, 37.9-93% (*A. parasiticus*). The mycelial dry weight of *A. flavus* and *A. parasiticus* was decreased up to 46.1-58.7%, 28.9-51.3% (Myrrh resin), and between 45-56.9%, 33.3-55.9% (Mahaleb seed extract). Nonetheless, the two plant extracts did not detoxify aflatoxin B_1 . Therefore, it apparent that the resin of *C. myrrha* and seed extract of *P. mahaleb* affected the biosynthesis pathway of aflatoxins. Thus, they can be recommended as effective natural plant biopreservative against aflatoxin contamination of food and feed products.

Keywords: Aflatoxigenic, *Aspergillus flavus*, *A. parasiticus*, *Commiphora myrrha*, *Prunus mahaleb*

1. Introduction

Commiphora myrrha (Nees) Engl. (syn. *C. molmol*), myrrh (Mor Hijazi, in Arabic) of the family Burseraceae, is small tree or large shrub which found in dry and arid regions of Ethiopia, Somalia, North Kenya, North Africa, and Middle East (Abd-Ulgadir *et al.*, 2015; Ali, 2007; Omer *et al.*, 2011; Su *et al.*, 2011). Myrrh gum-resin is the dried resinous exudate from plant stem of different *Commiphora* species. *C. myrrha* has various traditional uses in food and drink as flavoring, perfumes, and as fragrance in other cosmetics (Ali, 2007; Marshall, 2004). The natural gums are of high biocompatibility, available at low cost, low toxicity, and eco-friendly compared to the synthetic ones (Yusuf and Usman, 2011). Medicinally, myrrh gum has been extensively used for treatment of various diseases (El Ashry *et al.*, 2003; Shen and Lou, 2008), rheumatic complaints, tooth decay, gum disease, and helminth infection (Abd-Ulgadir *et al.*, 2015; Haffor *et al.*, 2010), antiseptic, carminative, anti-inflammatory, and tonic in dyspepsia (Omer *et al.*, 2011; Su *et al.*, 2012). It exhibited numerous biological activities as anti-inflammatory, antibacterial, antifungal, antimicrobial, antioxidant, hepatoprotective, smooth muscles relaxing, antimalarial, anticandidal, antischistosomal, larvicidal, molluscicidal, anticancer, and hypolipidemic effect (Al-Abdalall, 2013; Al-Daihan *et al.*, 2013; Ali *et al.*, 2008; Dolara *et al.*, 2000; Gadir *et al.*, 2014; Shen *et al.*, 2012; Shulan *et al.*, 2011). Its antimicrobial activity, food preservation, pharmaceuticals, alternative medicine and natural therapies has been reported by many authors (Abd-Ulgadir *et al.*, 2015). Antimicrobial activity against gram-positive organisms, *Candida albicans*, and other microorganisms was observed (Al-Daihan *et al.*,

2013; Dolara *et al.*, 2000; Omer *et al.*, 2011; Shuaib *et al.*, 2013).

Prunus mahaleb L. (sync. *Cerasus mahaleb* L. Mill.) of the family Rosaceae is known as English cherry, Rock cherry, St. Lucie cherry and “mahleb, mahaleb, mahlab” in Arabic which grown abundantly in West Asia, North Africa, Middle East, and sometimes found in Eastern and Central Europe (Leri *et al.*, 2012; Özcelik and Koca, 2012; Seyyednejad *et al.*, 2008; Shams and Schmidt, 2007). Various products from seed kernels and fruits of mahaleb tree have many uses as pleasing spice in patisseries mixed with flour for their special fragrance, home baking and candy industry (Özcelik and Koca, 2012). It has been used in folk medicine in various ailments as tonic for sensory organs, heart diseases, asthma, blood pressure, diabetes, swelling of stomach, relieving pains arising from liver, kidney swelling, anti-kidney stones, inflammation, oxidative stress diseases, gastrointestinal problem, diarrhea, and for scenting and preservation purposes (Gerardi *et al.*, 2010; Oskoueian *et al.*, 2012; Shams *et al.*, 2007). These plants are of significant potential in therapeutic applications against human pathogens including bacteria, fungi, and viruses (Holetz *et al.*, 2002; Perez, 2003; Syyednejad, 2008). All methanol extracts from different parts of mahaleb including flowers, leaves, branches, fruits, fruit stalks, seed and seed coat showed antibacterial and antifungal activities (Özcelik and Koca, 2012). The ethanolic extracts of mahaleb had antibacterial activity against *Proteus mirabilis*, *Bacillus anthracis*, and *Staphylococcus aureus* (Seyyednejad *et al.*, 2008).

Plant and animal products are prone to infestation with various mycotoxins producers *molds* (El-Nagerabi *et al.*, 2012, 2013; Herzallah 2009; Salim and Ahmad 2010; Wagacha and Muthomi, 2008). Of these mycotoxins, aflatoxins are the most hazardous contaminants associated with adverse effects on health (Kumar *et al.*, 2008). They are the most devastating pathogens of various crops and milks (Abdulkadir *et al.*, 2004; El-Nagerabi *et al.*, 2012; Elshafie *et al.*, 2002; Payne 1998; Santacrose *et al.*, 2008). Aflatoxin B₁ is mutagenic, teratogenic and carcinogenic secondary metabolites of *Aspergillus flavus*, *A. parasiticus*, *A. nominus* and *A. pseudotamarii* (El-Nagerabi *et al.*, 2013; Sidhu *et al.*, 2009).

Different plant extracts inhibit the fungal growth and aflatoxins production by *A. flavus* and *A. parasiticus* which suggest their antifungal and antiaflatoxigenic activities (El-Nagerabi *et al.*, 2012). Many plants were examined such as *Hibiscus sabdariffa* (Al-Shayeb and Mabrook, 1984; El-Nagerabi *et al.*, 2012), herbals (Gowda *et al.*, 2004), and *Garcinia cowa* and *G. pendunculata* fruits (Joseph *et al.*, 2005), *Syzygium aromaticum*, *Cucuma longa*, *Allium sativum*, and *Ocimum sanctum* (Reddy *et al.*, 2009). Essential oils from anise, caraway, cinnamon, black cinum, fennel plants, and *Negella sativa* displayed similar effects (Bullerman *et al.*, 1977; El-Nagerabi *et al.*, 2012; Farag *et al.*, 1989; Hasan, 1994; Maraqa *et al.* 2007; Montes-Belmont and Carvajal, 1998; Patkar *et al.*, 1993; Soher, 1999; Soliman and Badeaa, 2002). Investigations using different extracts from *Acacia seyal*, *Boswellia sacra*, *Balanites aegyptiaca*, *Moringa stenopetala*, *Tamaridus indica*, and *Adansonia digitata* revealed different inhibitory effects on aflatoxin secretion by aflatoxigenic fungi (El-Nagerabi *et al.*, 2012, 2013, 2016).

Different detoxification procedures have been tested for their inhibitory effect on the fungal growth and aflatoxin production (El-Nagerabi *et al.*, 2016; Oguz, 2011). Numerous chemicals and physical factors were evaluated for their decontamination properties (Kumar *et al.*, 2009). Nonetheless, safety issues and undesirable impacts on humans, animals and their environment restricted their use in food industries (Szczerbanik *et al.*, 2007; Vijayanandraj *et al.*, 2014). On the other hand, the biological detoxification of aflatoxin using different microorganisms was attempted by many authors (e.g. El-Nezami *et al.*, 1998; Shantha, 1999). Nonetheless, these microorganisms consumed nutrients from food for their growth and secrete toxic metabolites (Vijayanandraj *et al.*, 2014). Therefore, this supports the need for eco-friendly plant extracts as biocontrol method. However, the antifungal and detoxification property of *Commiphora myrrha* and *Prunus mahaleb* on the aflatoxigenic fungi was not tested. This study aiming at investigation of the effects of *C. myrrha* gum and *P. mahaleb* seed extract on two aflatoxigenic strains namely, *A. flavus* (SQU21) and *A. parasiticus* (CBS921.7) [NRRL22999]. The findings will build the knowledge on the bioactivities of these plants and their uses for the advancement in food and feed industries.

2. Materials and Methods

2.1 *Aspergillus Flavus* and *A. Parasiticus* Strains

Aspergillus flavus (SQU21) and *A. parasiticus* (CBS921.7)[NRRL22999] strains from previous study were used (El-Nagerabi *et al.*, 2016) as identified by Raper and Fennell (1965).

2.2 Collection and Characteristics of *C. Myrrha* Resin and Seeds of *P. Mahaleb*

The resin granules of *C. myrrha* and the seeds of *P. mahaleb* were purchased from the local market of Nizwa, Oman, and were stored at 25-33 °C. The resin from the myrrh stem is a yellow fragrant oleo-gum with aromatic

odour (Orwa *et al.*, 2009; Shuaib *et al.*, 2014; Shulan *et al.*, 2011). Ribose and galacturonic acid are the major constituents isolated from *C. myrrha* oleo-gum-resin (Ammar *et al.*, 2010). The oleo-gum resin contains about 2-8% volatile oil, 23-40% alcohol-soluble resin, 40-60% gum. Chemically it contains series of metabolites such as anti-inflammatory triterpenes (Carvalho *et al.*, 2008; Hanuš *et al.*, 2008; Refat *et al.*, 2011; Shuaib *et al.*, 2014; Shulan *et al.*, 2011). It decreases blood lipids and cholesterol and the guggulsterones act as antagonist ligands (Carvalho *et al.*, 2008). The oil composed of α -pinene, dipentene, limonene, cuminaldehyde, cinnamic aldehyde, eugeno, m-cresol, heerabolene, cadinene, sesquiterpene, abicyclic sesquiterpene, formic acid, acetic acid, myrrholic acid, and palmitic acid. The resin composed of acetate, 3-epi-lupenyl acetate, lupeone, 3-epi- α -amirin, α -amirone, acetyl β -eudesmol and a sesquiterpenoid lactone. It is a mixture of furanoeudesma-1,3-dien and lindestrene and dihydropyrocuzeren of a resinous myrrh odor (Mrongiu *et al.*, 2005). It contains about 15 amino acids and high yields of mixture sugars and acidic oligosaccharides, where in fractions of D-galactose, L-arabinose, and 4-methyl D-glucuronic acid were detected. Two aldobiuronic acids, which present as 6-O-(4-O-methyl- β -D-glucuronosyl)-D-galactose and 4-O-(4-O-methyl- α -D-glucuronosyl)-D-galactose were detected (Hanuš *et al.*, 2008; Soni *et al.*, 2013). Medicinally, it is used for treating various diseases and exhibited interesting biological activities such as anti-inflammatory, antibacterial, antifungal, antimicrobial, antimycobacterial, antioxidant, hepatoprotective, smooth muscle relaxing, antimalarial, anticandidal, antischistosomal, larvicidal, molluscicidal, anticancer, antiulcer and hypolipidemic effects (Ali *et al.*, 2008; Shulan *et al.*, 2011). The dry kernel of mahaleb contains 3.2% crude fat, 2.8% crude protein, 6.3% ash, 5.7% fiber, 82.0% soluble carbohydrates, and trace amounts of fats and protein (Herrera *et al.*, 1981). The seeds have high protein content (31%), cyanogenic glucoside and coumarins including herniarin (7-methoxycoumarin) and coumarin. It was found to contain 31% oil which is abundant in α -eleostearic (38.32%), oleic (31.29%), and linoleic (22.96%) (Sbihi *et al.*, 2014). In addition to tannins, traces of hydrocyanic acid, dihydrocoumarin and cyanogenic glucosides of amygdaline (mandelonitrile- β -gentio-bioside) (ÖZÇELİK *et al.*, 2012; Patton *et al.*, 1997; Jerković, 2011). The kernels contain 27-40% fatty oil with unusual composition of conjugated fatty acids including 9, 11, 13-octadecatrienoic acid, conjugated linoleic acid (38.81%), oleic acid (28.45%), linoleic acid (20.67%), palmitic acid (3.74%), stearic acid (2.25%) and arachidic acid (0.3%). Aliphatic hydrocarbons, alcohols, carbonyls, fatty acids (dodecanoic, tetradecanoic, hexadecanoic and linoleic acids), terpenes, C13-norisoprenoids and phenylpropane derivatives (Coumarin 0.3-2.4%) were detected (Jerković, 2011). Terpenes, norisoprenoids and benzene derivatives, minor percentages of aliphatic compounds and furan derivatives were extracted (Oral, 2014).

2.3 Growth of *Aspergilli* Strains on Media Enriched with Myrrha Resin and Mahaleb Extract

The strains of *A. flavus* and *A. parasiticus* were grown on Potato Dextrose Agar (PDA) and incubated for 7 days at ambient temperature (25-32 °C). Glass tubes of 5 mm in diameter were sterilized and used to cut several discs from each of the growing fungal colonies. Inoculum from the growing colonies were added to 250 flasks containing 200 ml of yeast malt broth with different concentrations of *myrrh* resin (0.0%, 1.0%, 1.25%, 2.5% and 3.25% w/v), and *mahaleb* extract (0.0%, 0.75%, 1.5%, 2.5%, and 3.5% w/v). Three replicates were incubated at 25-32°C for 15 days. Other sets were kept to measure the dry weight of the fungal mycelia using Oven method.

2.4 Effect of the Extracts on Synthetic Aflatoxin B₁

Pure aflatoxin B₁ of 885 ppb concentration was prepared in 100 ml sterile distilled water. The highest concentrations of resin (3.25%) and mahaleb (3.5%) were separately added to flasks containing pure aflatoxin B₁. As a control, flask containing aflatoxin B₁ was left without any extract. The flasks were incubated at 25-32°C for 7 days and aflatoxin concentrations were assessed.

2.5 Extraction and Assay of Aflatoxin

Alfa Test-P Affinity method was used for aflatoxin extraction and detection as described by many authors and adopted in our previous study (El-Nagerabi *et al.*, 2012). To the 200 ml fungal culture, 5g of sodium chloride in addition to 100 ml methanol:water (70:30 V/V) as extraction solution were added. To the filtrate, 15 ml distilled water were added, mixed, filtered with glass microfilters. Ten ml from the diluted filtrate were passed via Afla-Test-P Affinity Column and the column was cleaned by 10 ml distilled water. The extracted aflatoxin was eluted with one ml methanol (HPLC grade) and one ml of AflaTest developer was added to elute in the cuvette, and vortexed. The aflatoxin concentration was measured by calibrated Vicam fluorometer (Series-4EX) (El-Nagerabi *et al.*, 2016; Elshafie and Al-Shally, 1998).

2.6 Statistical Analysis

One-way ANOVA test (correlation coefficient) under SPSS software (version 11.0) was used to determine the

variation between the effects of different concentrations of *C. myrrha* resin gum and *P. mahaleb* seed extract on aflatoxin inhibition-detoxification and fungal growth.

3. Results and Discussion

3.1 Effect of Myrrh Resin and Mahaleb Extract on Fungal Growth and Aflatoxin Secretion

Worldwide, researchers evaluating the uses of different plant products and microorganisms for biological control of aflatoxigenic molds (ex: Reddy *et al.*, 2009; Shantha, 1999; Suleiman *et al.*, 2008). Numerous herbals, medicinal and aromatic plants were screened for their antifungal properties (El-Nagerabi *et al.*, 2012, 2013, 2016; Gandomi *et al.*, 2009; Maraqa *et al.*, 2007; Montes-Belmont and Carvajal, 1998; Patker *et al.*, 1993; Soher, 1999; Soliman and Badeaa, 2002). In the present studies, the effect of *C. myrrha* resin, *P. mahaleb* seed extract on the aflatoxigenic *Aspergillus flavus* and *A. parasiticus* was evaluated. The results showed that the total aflatoxin produced by the two *Aspergillus* strains was significantly ($p < 0.05$) inhibited by different concentrations of resin (1, 1.25, 2.5, and 3.25g/100 ml). The total aflatoxin was decreased by 51.9-95.7% (*A. flavus*) and 46.9-92% (*A. parasiticus*) (Fig. 1), and aflatoxin B (B_1 and B_2) was inhibited by 51.7-93.5% and 39-89.7%, respectively (Fig. 2). The mycelial dry weights of the two species were significantly ($p < 0.05$) decreased with concentrations of resin (Fig. 3). The mycelial dry weight was decreased by 46.1-58.7% (*A. flavus*), and 28.9-51.3% (*A. parasiticus*). On the other hand, the total aflatoxin was significantly ($p < 0.05$) inhibited by all tested concentrations of mahaleb seed extracts (0.75, 1.5, 2.5, 3.5g/100 ml) compared to the control. The total aflatoxin inhibition ranged between 53.7-95.6% for *A. flavus*, and 40-94.7% for *A. parasiticus* (Fig. 4), whereas aflatoxin B inhibition was 50-93.6% for *A. flavus* and decreased by 37.9-93% for *A. parasiticus* (Fig. 5). The mycelial dry weight decreased by 45-56.9% for *A. flavus*, and 33.3-55.9% for *A. parasiticus* (Fig. 6).

About 50% of *Aspergilli* are aflatoxin producers including the two strains used in this study (El-Nagerabi *et al.*, 2016). There are some investigations on the uses of myrrh resin for diseases treatment (Abd-Ulgadir *et al.*, 2015; Al Ashry *et al.*, 2003; Haffor, 2010; Shen and Lou, 2008), antiseptic carminative, and anti-inflammatory tonic in dyspepsia (Omer *et al.*, 2011), various biological activities such as antibacterial, antifungal (Al-Abdalall, 2013; Al-Daihan *et al.*, 2013; Ali *et al.*, 2008; Dolara *et al.*, 2000; Gadir *et al.*, 2014; Shen *et al.*, 2012; Shulan *et al.*, 2011), as food preservative (Abd-Ulgadir *et al.*, 2015), and antimicrobial activity against gram-positive bacteria, *Candida albicans*, and other microorganisms (Al-Daihan *et al.*, 2013; Dolara *et al.*, 2000; Omer *et al.*, 2011; Shuaib *et al.*, 2013). On the other hand, *P. mahaleb* used as spice in home baking (Özcelik and Koca, 2012), folk medicine (Gerardi *et al.*, 2010; Oskoueian *et al.*, 2012; Shams *et al.*, 2007), and against human pathogenic bacteria, fungi, and viruses (Holetz *et al.*, 2002; Perez, 2003; Syyednejad, 2008). Extract from different plant parts showed antibacterial and antifungal properties (Özcelik and Koca, 2012), and antibacterial activities against *Proteus mirabilis*, *Bacillus anthracis*, *Staphylococcus aureus* (Seyyednejad *et al.*, 2008). The effect of *C. myrrha* resin and *P. mahaleb* extracts on aflatoxigenic molds was not investigated yet. This encouraged the need for testing their inhibitory nature. The *C. myrrha* resin of between 1-3.25% resulted in 51.9-95.7% inhibition of total aflatoxin production (*A. flavus*) and 46.9-92% (*A. parasiticus*). Aflatoxin B (B_1 and B_2) was decreased by 51.7-93.5% for *A. flavus* and 39.5-89.7% for *A. parasiticus* strain. Similarly, *P. mahaleb* seed extracts inhibited the total aflatoxin up to 53.7-95.6% for *A. flavus*, and 40-94.7% for *A. parasiticus*, whereas aflatoxin B inhibition was 50-93.6% and 37.9-93%, respectively. In similar studies using different plant extracts showed apparent inhibition of the fungal growth and aflatoxin production by aflatoxigenic fungi. *Syzgium aromaticum*, cinnamon, *Curcuma longa*, *Allium sativum*, *Ocimum sanctum*, *Garcinia cowa*, *A. digitata* (baobab), *Boswellia sacra*, *Tamaridus indica* and *Hebiscus sabdariffa* effectively inhibit the growth of *A. flavus* and aflatoxin production (Al-Shayeb and Mabrook, 1984; Bullerman *et al.*, 1977; El-Nagerabi *et al.*, 2012, 2013; Joseph *et al.*, 2005; Reddy *et al.*, 2009). The present results showed that the highest inhibition (92-95.7%, 94.7-95.6%) at 3.25% myrrh resin, and 3.5% mahaleb. These findings point the high possibility for the presence of various aflatoxin inhibitors in myrrh resin and mahaleb seed extract which affect the biochemical synthesis of aflatoxin. These chemicals are responsible for the biological properties of these plant extracts (Büchele, *et al.*, 2003; El-Nagerabi *et al.*, 2012, 2013, 2016; Safayhi and Sailer, 1997; Singh *et al.*, 2008;). On the other hand, the addition of different concentrations of *C. myrrha* resin and *P. mahaleb* seed extract to the yeast malt broth inoculated with the two strains, evidently inhibited their growth performance (Fig. 3, 6). Similarly, *C. myrrha* extracts inhibited the growth of both bacterial and fungal standard species (Abd-Ulgadir *et al.*, 2015; Omer, *et al.*, 2011), whereas the oil of *C. myrrha* and *C. molmol* showed antibacterial and antifungal activities and inhibited the growth of *Aspergillus flavus*, *A. niger* and *Penicillium citrinum* (Al-Abdalall, 2013; Al-Daihan *et al.*, 2013; Ali, 2007; Dolara *et al.*, 2000; Gadir and Ahmed, 2014; Shuaib *et al.*, 2013). Extract from different parts of *P. mahaleb* showed inhibitory effect against gram-positive, gram-negative bacteria and fungal standard strains (Özcelik and Koca, 2012; Seyyednejad *et al.*, 2008). On the contrary, the fungal growth was enhanced by the high nutritive

extract from fruit of *Balanites aegyptiaca* and *Tamarindus indica* (El-Nagerabi *et al.*, 2013). On the other hand, different concentrations of calyx extract (5-12.5%) from *H. sabdariffa* did not inhibit or enhance the mycelial growth of *Aspergillus* species (El-Nagerabi *et al.*, 2012). Other studies showed different effects on the mold growth and aflatoxin production (Bullerman *et al.*, 1977; Guerin and Reveillere, 1984; Joseph *et al.*, 2005; Reddy *et al.*, 2009). Therefore, it is evident that *C. myrrha* resin and *P. mahaleb* seed extracts contains different chemical inhibitors which affect the biochemical synthesis of aflatoxin as concluded in many studies (Büchele *et al.*, 2003; Da Costa *et al.*, 2010; El-Nagerabi *et al.*, 2012, 2013, 2016; Safayhi and Sailer, 1997; Singh *et al.*, 2008).

3.2 Detoxification of Aflatoxin B₁ by Resin of Myrrh and Seed Extract of Mahaleb

The natural plant extracts are biologically safe and ecofriendly for detoxification comparable to the other methods (Alberts *et al.*, 2009; El-Nagerabi *et al.* 2012, 2013, 2016; Kumar *et al.* 2009; Oguz, 2011; Prakash *et al.*, 2011). The ability of different herbal, medicinal and aromatic plants as biodegraders to aflatoxin has been reported (Sandoskumar *et al.*, 2007). Root extracts of garlic (*Allium sativum*) and onion (*Allium cepa*) degrade aflatoxin B₁ up to 58.5% (Velazhahan *et al.*, 2010). *Trachyspermum ammi* seed extract degrades 90% of aflatoxin G₁ by modification of lactone ring in the toxin (Velazhahan *et al.*, 2010). Medicinally, *C. myrrha* resin has been used for treatment of various diseases (Al Ashry *et al.*, 2003; Shen and Lou, 2008); rheumatic complaints, tooth decay, gum disease, and helminth infection (Haffor *et al.*, 2010; Abd-Ulgadir *et al.*, 2015), antiseptic, carminative, anti-inflammatory, and tonic in dyspepsia (Omer *et al.*, 2011). It showed many biological activities as antibacterial, antifungal, antimicrobial, antimalarial, anticandidal, antischistosomal, larvicidal, and molluscicidal (Al-Abdalall, 2013; Abd-Ulgadir *et al.*, 2015; Al-Daihan *et al.*, 2013; Ali *et al.*, 2008; Dolara *et al.*, 2000; Gadir *et al.*, 2014; Shen *et al.*, 2012; Shulan *et al.*, 2011). Antimicrobial activity against *Candida albicans*, and other microorganisms was reported (Al-Daihan *et al.*, 2013; Dolara *et al.*, 2000; Omer *et al.*, 2011; Shuaib *et al.*, 2013). On the other hand, *P. mahaleb* has been used in many folk medicine and preservation purposes (Gerardi *et al.*, 2010; Oskoueian *et al.*, 2012; Shams *et al.*, 2007). It is used against human pathogenic bacteria, fungi, and viruses (Holetz *et al.*, 2002; Perez, 2003; Syyednejad, 2008). Alcohol extract from different part of the plant showed antibacterial and antifungal activities (Özcelik and Koca, 2012). It showed antibacterial activity against *Proteus mirabilis*, *Bacillus anthracis*, and *Staphylococcus aureus* (Seyyednejad *et al.*, 2008). In the present investigations, 3.25% (w/v) of *C. myrrha* resin and 3.5% (v/v) of *P. mahaleb* had no significant effect on synthetic aflatoxin, which indicates the lack of detoxification properties compared to their inhibitory effects on aflatoxin production, and fungal growth as concluded by many authors (e.g. Da Costa *et al.*, 2010; El-Nagerabi *et al.*, 2016; Paranagama *et al.*, 2003; Sandoskumar *et al.* 2007).

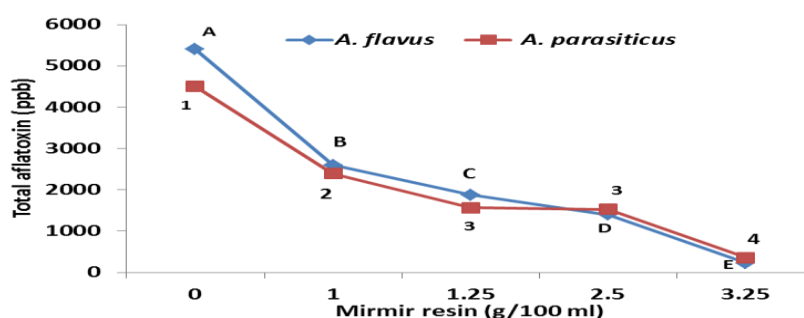


Figure 1. Total aflatoxin production of *A. flavus* strain SQU21 and *A. parasiticus* strain CBS921.7 at different concentrations of *Commiphora myrrha* resin extract (Identical numbers and letters indicate no significant difference, $p < 0.05$)

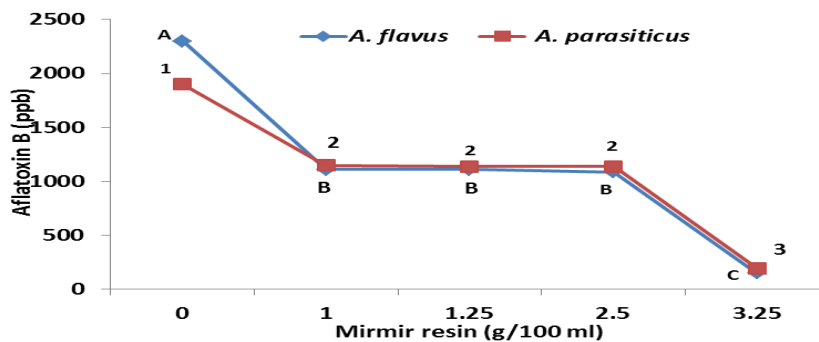


Figure 2. Aflatoxin B production of *A. flavus* and *A. parasiticus* strains at different concentrations of *C. myrrha* resin extract (Identical numbers and letters indicate no significant difference, $p < 0.05$)

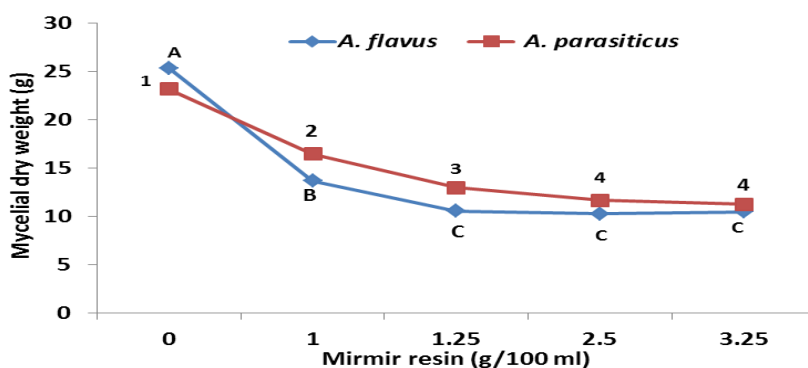


Figure 3. Mycelial dry weight of *A. flavus* and *A. parasiticus* strains at different concentrations of *C. myrrha* resin extract (Identical numbers and letters indicate no significant difference, $p < 0.05$)

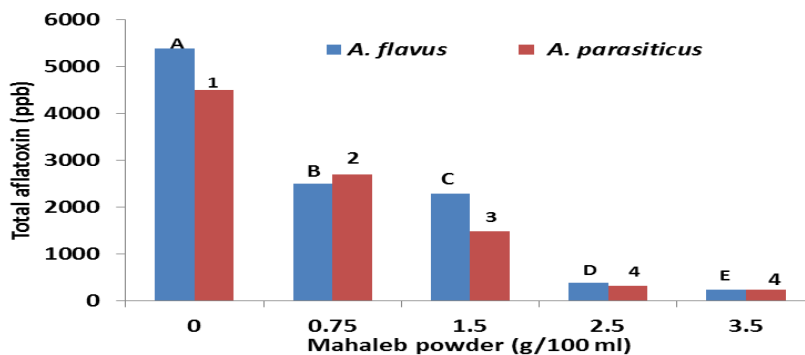


Figure 4. Total aflatoxin secretion by *A. flavus* and *A. parasiticus* strains at different concentrations of *Prunus mahaleb* (Similar numbers and letters indicate showed no significant difference, $p < 0.05$)

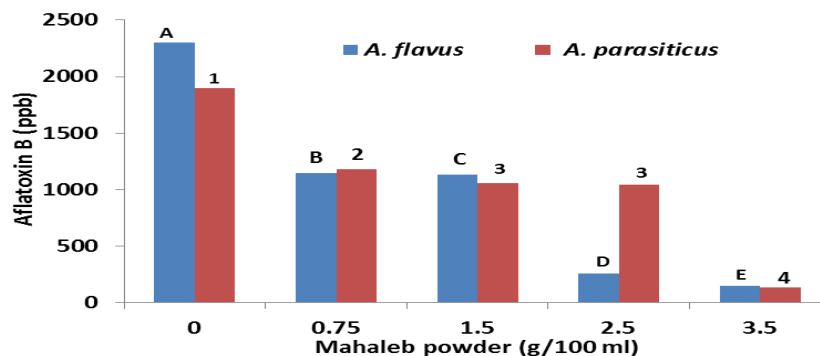


Figure 5. Aflatoxin B secretion of by *A. flavus* and *A. parasiticus* under different concentrations of *P. mahaleb* seed extract (Different numbers and letters indicate significant difference, $p < 0.05$)

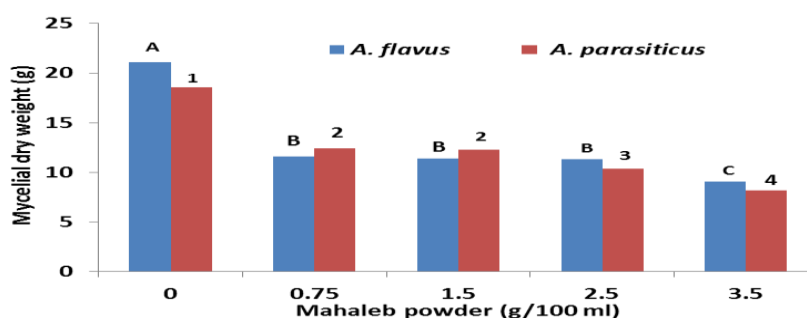


Figure 6. Mycelial dry weight of *A. flavus* and *A. parasiticus* at different concentrations of *P. mahaleb* extract (Different numbers and letters indicate significant difference, $p < 0.05$)

4. Conclusion

We screened the biological activities of different concentrations of *C. myrrha* resin and *P. mahaleb* seed extract on the growth and aflatoxin production by *A. flavus* (SQU21) and *A. parasiticus* (CBS921.7). These two plant extracts evidently reduce aflatoxin production and the fungal growth which may suggests the inhibitory effect to aflatoxin biochemical synthesis pathway. None of the two extracts detoxify pure aflatoxin B₁ as suggested by many researchers (Abulmajeed, 2011; Banno *et al.*, 2006; El-Nagerabi *et al.*, 2012, 2013, 2016; Gupta *et al.*, 2001; Langmead and Rampton 2006; Miller & Morris, 2004; Mothana *et al.* 2011; Suhail *et al.* 2011). Therefore, toxicity of biologically active chemical components which reduce aflatoxin production needs more attention. This will build the data on their applications in food preservation industry and pharmaceutical activities.

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