The Potential of Baobab (*Adansonia digitata* L.) Extracts as Biocontrol on the Growth and Aflatoxin Production by *Aspergillus flavus* and *A. parasiticus*

Saifeldin A. F. El-Nagerabi¹, Abdulkadir E. Elshafie², Suleiman S. AlKhanjari¹, Saif N. Al-Bahry² & Mohamed R. Elamin³

¹ Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, Oman
² Department of Biology, College of Science, Sultan Qaboos University, Oman
³ Department of Chemistry, Sudan Academy of Science, Khartoum North, Sudan

Correspondence: Saifeldin A. F. El-Nagerabi, Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, Birkat Al Mouz, Nizwa, P. O. Box 33, PC 616, Oman. Tel: 968-9636-5051. E-mail: nagerabi@unizwa.edu.om; nagerabi@hotmail.com

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Abstract

Moulds and associated mycotoxins, especially aflatoxins, are important factors that adversely affect food and feed produced from contaminated plant and animal products. They are lethal to humans and animals, which emphasizes the great concern in food and feed production. In this study, the effects of baobab (*Adansonia digitata*) extracts on the vegetative growth and aflatoxin secretion by *A. flavus* (SQU21) and *A. parasiticus* (CBS921.7) strains were examined. Different concentrations of baobab fruit extract (1.5, 3, 5, and 7% w/v) and essential oil (0.5, 1, 3 and 5% v/v) was used. Fruit extract of baobab 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 between 29.9-79.2% and 13-68% for the two strains, respectively. 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 baobab seeds. The two extracts significantly reduced the vegetative growth and the mycelial dry weights of selected fungi. This indicates the antifungal activity and inhibitory effect of baobab on the growth and aflatoxin production by the two toxigenic strains. Thus, fruit extract and essential oil of *A. digitata* can be suggested as potentially effective biocontrol and biopreservative substrates against food and feed contamination by aflatoxigenic moulds.

Keywords: *Adansonia digitata*, *Aspergillus flavus*, *A. parasiticus*, baobab fruit, essential oil

1. Introduction

*Adansonia digitata* L. (Baobab) of the family Malvaceae is a large iconic deciduous and stem-succulent tree indigenous to the dry regions of Africa. It is found in many countries of South Africa (Zimbabwe, Mozambique, South Africa), West Africa (Mali, Benin, Senegal, the Ivory Cost, Cameroon, Burkino Faso), and East Africa (Kenya, Uganda, Sudan, Tanzania) (Sidibé & Williama, 2002; Wickens & Lowe, 2008; Kamatou, VermaaK, & Viljoen, 2011; Vermaak, Kamatou, Komane-Mofokeng, Viljoen, & Beckett, 2011; De Smedt, Sanchez, Van den Bilcke, Simbo, Potters, & Samson, 2012). In the past decade, different parts of the baobab tree have been reported to be useful and this has attracted the interest of pharmaceutical companies and scientists. This is due to its various traditional uses as medicinal, nutritional and cosmetic plant (Igboeli, Addy, & Salami, 1997; Wickens & Lowe, 2008; Buchmann, Prechsler, Hartl, & Vogl, 2010; Kamatou et al., 2011). Recently, the European Commission authorized the importation of baobab fruit pulp as a novel food for human consumption (Buchamann et al., 2010). In 2009, it was approved by the Food and Drug Adminstration (FDA) as a food ingredient in the United States of America (Addy, 2009). The dry pulp is commonly used to prepare fruit juice with higher levels of vitamin C than orange, and calcium than milk (Assogbadjo, Chadare, Kakari, Fandohan, & Baidu-Forson, 2012). Various plant parts such as leaves, bark, and fruit pulp have been traditionally used as immuno-stimulant, anti-inflammatory, analgesic, and pesticide, and in the treatment of fever, diarrhoea, cough, dysentery, haemoptysis, tuberculosis, microbial infection and worms (Wickens & Lowe, 2008; Kamatou et al.,...
The seeds are used as roasted snacks, fermented and used as a thickening and flavouring agent in soup (Igboeli et al., 1997). The oil extracts are used as food, fuel, medicine, cosmetic applications and topical treatment of various conditions such as dandruff, muscle spasms, varicose veins and wounds (Chivandi, Davidson, & Erlwanger, 2008; Kamatou et al., 2011; Vermak et al., 2011).

Mycotoxins are toxic secondary metabolites of fungal origin and natural contaminant of agricultural commodities under both pre- and post-harvest conditions (Wagacha & Muthomi, 2008; Herzallah, 2009; Salim & Ahmad, 2010). The species of the genus Aspergillus, Fusarium, and Penicillium are the major mycotoxin producing fungi. The most important mycotoxins are aflatoxins, fumonisins, and ochratoxins (Kumar, Basu, & Rajendran, 2008). Aflatoxigenic fungi are the most devastating contaminants of different plants and animals products (Payne, 1998; Elshafie, Al Rashdi, Al-Bahry, & Bakheit, 2002; Abdulkadir, Al-Ali, Al-Kildi, & Jedah, 2004; Santacrose, Conversano, Casalino, Lai, Zizzadoro, & Centoducati, 2008; El-Nagerabi, Al-Bahry, Elshafie, & AlHilali, 2012). Aflatoxins in general and aflatoxin B₁, in particular are mutagenic and hepatocarcinogenic secondary metabolites secreted by Aspergillus flavus, A. parasiticus, A. nomius and A. pseudotamorii are pose serious effects on human and animal health (Sidhu, Chandra, & Behl, 2009; Elshafie, ElMubarak, El-Nagerabi, & Elshafie, 2010; Liu & Wu, 2010; El-Nagerabi et al., 2012).

The vegetative growth and associated aflatoxin production by A. flavus and A. parasiticus were found to be affected by many extracts from different plant parts due to their fungicidal and fungistatic properties (Soliman & Badeea, 2002; El-Nagerabi et al., 2012). This includes dry leaves and calyx extracts of Hibiscus sabdarifff (Al-Shayeb & Mabrook, 1984; El-Nagerabi et al., 2012), herbal compounds (Gowda, Malathi, & Suganthi, 2004), and fruit rinds of Garcinia cowa and G. pendunculata (Joseph, Jayaprakasha, Seli, Jena, & Sakariah, 2005). Leaf extract from Syzigium aromaticum, Cucuma longa, Allium sativum, and Ocimum sanctum showed significant antifungal activities and inhibit aflatoxin B₁ production by A. flavus and A. parasiticus (Reddy, Reddy, & Muralidharan, 2009). Similar effects were observed with essential oils from medicinal and herbal plants such as anise, caraway, cinnamon, black cinum, and fennel (Bullerman, Lieu, & Seier, 1977; Farag, Daw, & Abo-Raya, 1989; Soher, 1999; Patkar, Shetty, Poster, & Lacey, 1993; Hasan, 1994; Montes-Belmont & Carvajal, 1998; Soliman & Badeea, 2002). Oil of Nigella sativa at concentration of 1-3% completely inhibited aflatoxin production (Maraqa, Alsharoa, Farah, Albjeirami, Shakya, & Sallal, 2007; El-Nagerabi et al., 2012).

Many researchers worldwide are continuously assessing different detoxification methods and inhibition techniques on aflatoxin secretion by aflatoxigenic fungi (Gandomi, Misaghi, Basti, Bokaei, Khosravi, Abbasifar, & Javan, 2009; Kumar, Shukla, Singh, & Dubey, 2009; Oguz, 2011; El-Nagerabi et al., 2012). Reduction or inactivation of aflatoxin by various decontamination procedures using different physical and chemical methods have been studied extensively together with microbiological degradation (Alberts, Gelderblom, Botha, & Van Zyl, 2009; Kumar et al., 2009). Nevertheless, these synthetic chemicals are hazardous to humans and domestic animals as well as the environment (Szczesniak, Jobling, Morris, & Hoflind, 2007; Gandomi et al., 2009; Kumar et al., 2009; Prakash, Shukla, Sigh, Mishra, Dubey, & Kharwar, 2011). This prompted us to search for simple, safe, and environment friendly antifungal and growth inhibitors from biological sources. Nonetheless, the antifungal, inhibitory, and detoxification effects of A. digitata extracts on the fungal growth and aflatoxin production had not been screened. Thus, there is high potential for extracts from A. digitata to inhibit the fungal growth and aflatoxin production by these aflatoxigenic fungi. The present investigations aim to evaluate the effects of fruit pulp powder and oil extracted from seeds of baobab on the fungal growth and aflatoxin secretion of two aflatoxigenic strains of A. flavus (SQU21) and A. parasiticus (CBS921.7). This will contribute with international efforts to fill the gap in our knowledge about the antimicrobial properties of baobab and possibly lead to developments in the food industry related to preparation, preservation, storage, and consumption.

2. Materials and Methods

2.1 Fungal Isolates

Two strains of high aflatoxin-producer fungi of Aspergillus flavus (SQU21) and A. parasiticus (CBS921.7) [NRR22999] were obtained from the culture collections of Sultan Qaboos University, Oman. These isolates were cultivated on Czapek Dox Agar (CDA) and described taxonomically using the manual prepared by Raper & Fennel (1965). These strains were used as inoculum in this study.

2.2 Source and Properties of Adansonia digitata Extracts

The fruit powder of A. digitata pulp was purchased from AlNaser Company, Khartoum, Sudan. Numerous studies were carried on the nutritional constituents of baobab parts (Sidiib & Williams, 2002; Chadare, Houhouisgan, Linnemann, Nout, & Van Boekel, 2009; Assogbadjo et al., 2011). Biochemical analysis indicated that baobab parts (pulp, leaves and seeds) are rich in several microelements such as iron, vitamin C, A, E and F.
in addition to calcium, potassium, magnesium, zinc, proteins and lipids (Chadare et al., 2009). The oil extract of this plant was obtained from Chemistry for Life Company, Muscat, Oman. The chemical nature of the essential oil extracted from the seeds was reported by researchers. The oil is extremely stable with a half life of between 2 to 5 years, a high saponification value compared to other edible oils, and the iodine value is 87.9 g/100 g as non-drying oil, with 33% saturated, 36% monosaturated and 31 polysaturated fatty acids in addition to palmitic and oleic acids as major constituents (Vermaak et al., 2011).

2.3 Inoculation of Aspergillus Starins on Media Containing A. digitata Extracts

A. flavus (SQU21) and A. parasiticus (CBS921.7) were inoculated onto Potato Dextrose Agar (PDA) and incubated at ambient temperature of 25 ± 2°C for 10 days. Sterile thin glass tubes of 5 mm in diameter were used to cut several discs from the growing cultures. Two discs of 5 mm in diameter were added aseptically to each flask containing 200 ml sterile yeast malt broth with 1.5, 3, 5, and 7 g/100 ml of Adansonia digitata fruit pulp extract and 0.5, 1, 3 and 5 ml/100 ml oil extract. As a control, fruit pulp extract and oil extract were mixed with yeast malt broth and without any fungal inoculation. Three inoculated flasks from each treatment were incubated at 25 ± 2°C for two weeks. Similarly inoculated flasks were used to determine the mycelial dry weight of the two fungal strains.

2.4 Effect of A. digitata Fruit Extract and Oil on Pure Aflatoxin B1

Aflatoxin B1 powder (Sigma Company) was added to 100 ml sterile distilled water which gave an aflatoxin B1 concentration of 870 ppb. The highest concentrations from A. digitata fruit pulp (7 g/100 ml) and oil (5 ml/100 ml) were chosen. For this, 7 grams of A. digitata fruit pulp and 5 ml of oil were added to the different flasks of aflatoxin B1. The flasks were incubated at 25 ± 2°C for 10 days. The aflatoxin concentration was measured.

2.5 Extraction and Detection of Aflatoxin by Afla Test-P Affinity Column

For aflatoxin extraction, similar method used in our previous study on the effect of Hibiscus sabdariffa extract and Nigella sativa oil on the growth and aflatoxin B1 of Aspergillus flavus and A. parasiticus strains was adopted (El-Nagerabi et al., 2012). For measuring the concentration of aflatoxin, calibrated Vicam fluorometer (Series-4EX) from Vicam Company, Milford, MA, USA was used. The fluorometer was set at excitation wavelength of 360 nm and emission wavelength of 440 nm (Elshafie & Al-Shally, 1998).

2.6 Statistics and Data Analysis

To assess the variation between the effects of fruit and oil extracts of A. digitata extracts on the vegetative and aflatoxin production, one way ANOVA test (correlation coefficient) was used. The statistical package software SPSS (version 11.0) was used.

3. Results and Discussion

3.1 Effects of Fruit Pulp Extract of Baobab on Fungal Growth and Aflatoxins Production

The effects of various concentrations of Adansonia digitata (Baobab) fruit pulp extract on the total aflatoxin (Figure 1a), aflatoxin B1 (Figure 1b), and mycelia dry weight (Figure 1c) of A. flavus (SQU21) and A. parasiticus (CBS921.7) were recorded. The total aflatoxins and aflatoxin B1 production by the two Aspergillus strains were significantly (p < 0.05) reduced by the tested concentrations of baobab fruit extract (1.5, 3, 5, and 7 g/100 ml) compared to the control. Similarly, the mycelial dry weight of the two fungal strains was significantly (p < 0.05) reduced by the different concentrations of baobab fruit pulp extract comparable to the control.

The antifungal activities and detoxification properties of different plant extracts were investigated by many researchers (Gandomi et al., 2009; Kumar et al., 2009; Oguz, 2011; El-Nagerabi et al., 2012). Nonetheless, based on the available literature, the antifungal ability and detoxification properties of fruit extract of A. digitata on the fungal growth and aflatoxins production by Aspergillus species had not been evaluated before. To our knowledge, this is the first study on the biological activities of different extracts from this plant. However, extracts from fruit rind of Garcinia cowa and G. penduculata completely inhibited the growth and aflatoxin B1 production by A. flavus (Joseph et al., 2005). Therefore, it is possible that fruit and other extracts from A. digitata could reveal similar inhibitory effects on the fungal growth and aflatoxins secretion by the two aflatoxigenic strain of A. flavus and A. parasiticus. Hence, it is evidently important to evaluate the inhibitory effect of various extracts from A. digitata against the fungal growth and aflatoxin production by aflatoxigenic fungi and compared with the similar studies which used different extracts from herbal and medicinal plants. In the present investigations, the concentrations of baobab fruit extract (1.5-7%) apparently inhibited total aflatoxin production by 20.4-68.5% for A. flavus (SQU21) and 11.9-69.1% for A. parasiticus (CBS921.7), whereas the inhibition of aflatoxin B1 production ranged between 29.9-79.2% and 13-68% for the two strains as suggested by Joseph et al. (2005).
using similar extract from fruit rind of G. cowa and G. penduculata. Also neem seed cake and leaf extract of Azadirachta indica inhibited the fungal growth and aflatoxin production by A. flavus and A. parasiticus. Other studies showed similar inhibition of the fungal growth and aflatoxin production. For example, aqueous extracts from mature leaves of Vernonia amygdalina, Sena elata and Cymbopogon citratus (Suleiman, Emua, & Taiga, 2008), plant extract of Syzygium aromaticum, Curcuma longa, Allium sativum and Ocimum sanctum (Reddy et al., 2009), herbal compounds (Gowda et al., 2004), and dry leaves and calyx extracts of Hibiscus sabdariffa (A-Shayeb & Mabrook, 1984; El-Nagerabi et al., 2012). Cinnamon extract concentrations of 0.02-20% inhibit aflatoxin production by 25-100%, and 2% of cinnamon led to 97% inhibition of aflatoxin secretion by aflatoxigenic fungi (Burman et al., 1977). About 91.5-97.9% reduction in aflatoxin B₁ production by A. flavus and A. parasiticus was caused by leaf and calyx extracts (5-12.5%) of H. sabdariffa (El-Nagerabi et al., 2012; Al-Shayeb & Mabrook, 1984). Our results showed that the highest inhibition levels of total aflatoxin (68.5-69.1%) and aflatoxin B₁ (68-79.1%) were reported at 7% concentration of baobab fruit extract. Therefore, it is possible that various growth inhibitors present in this plant extracts would affect aflatoxin secretion by aflatoxigenic fungi. On the other hand, inoculation of A. flavus (SQU21) and A. parasiticus (CBS921.7) strains on yeast malt broth containing different concentrations of baobab fruit extract (1.5, 3, 5, and 7 g/100 ml) significantly inhibited the fungal growth and mycelial dry weights of the two strains. Similarly, extract from the dried leaves of H. sabdariffa evidently retarded the growth and vigour of different fungi (Guerin & Revillere, 1984). On the contrary, calyx extract (5-12.5%) from H. sabdariffa did not show any effect on the mycelial growth of Aspergillus species (El-Nagerabi et al., 2012). Some herbal drugs and medicinal plants inhibit the mycelial growth of A. flavus and A. parasiticus while others improved mycelial growth, but retarded aflatoxin secretion (Bahk & Marth, 1983; Gowda et al., 2004; Joseph et al., 2005; Suleiman et al., 2008; Reddy et al., 2009; Da Costa et al., 2010). Cinnamon at the concentrations of between 0.02-2.0% inhibited aflatoxin biosynthesis and the growth of A. parasiticus by 16-100% (Burman et al., 1977). The leaf extracts cassia and bay enhance the mycelial growth of A. parasiticus and inhibit the fungal growth and aflatoxin production by A. flavus (Paranagama, Abeyesekera, Abeywickrama, & Nugaliyadde, 2003; Krishnamorthy, & Shashikala, 2006; Sandosskumar, Karthikeya, Mathiyazhaga, Mohankumar, Chandrasekar, & Velazhahan, 2007). Therefore, it is evident that A. digitata fruit extract showed antifungal activities and inhibitory effect on aflatoxin production by A. flavus and A. parasiticus.

3.2 Effects of Essential Oil of Baobab on Fungal Growth and Aflatoxins Production

The uses of essential oils (EOs) extracted from herbal, medicinal and aromatic plants against the fungal growth and aflatoxin production of A. flavus and A. parasiticus have been suggested by many researchers (Maraqa et al., 2007; El-Nagerabi et al., 2012). They had different fungistatic activities (Gandomi et al., 2009; Shukla et al., 2012). Nigella sativa oil at 3% completely inhibited (Maraqa et al., 2007). At concentrations of 1-3%, this oil caused 47.9-58.3% reduction in aflatoxin B₁ for A. flavus and 32-48% for A. parasiticus strains (El-Nagerabi et al., 2012). Oil of cassia and bay leaves reduced aflatoxin B₁ (98%) and stimulated fungal growth, whereas coriander oil had no effect on the fungal growth and its toxigenicity (Attanda, Akqan, & Oluwafemi, 2007). Aflatoxin B₁ production by NKD-208 isolates of A. flavus was strongly inhibited at lower fungistatic concentrations of essential oil of Callistemon lanceolatus (Shukla et al., 2012). All concentrations of Zataria multiflora essential oil exhibited significant inhibition of fungal growth as well as spore production (Gandomi et al., 2009). Ocimum gratissimum oil shows better efficacy as a fungitoxicant than prevailing fungicide Wettasul-80 (Prakash et al., 2011). The essential oils of T. ericocalyx and T. x-porlock were evidently fungicidal and inhibitory to aflatoxin production (Rasool & Abyaneh, 2004). Of the 96 plant extracts, EOs proved to be the most effective extract controlling aflatoxigenic strains (Bluma, Amaiden, & Etcheverry, 2008). Frankincense of B. carteri at 2% (v/v) showed the strongest mycelium inhibition against A. flavus and other pathogenic fungi (Udomsilp et al., 2009). In this investigation, the effects of different concentrations of A. digitata essential oil (0.5, 1, 3, and 5 ml/100 ml) on total aflatoxin secretion (Figure 2a), aflatoxin B₁ (Figure 2b) and the mycelial growth (Figure 2c) of A. flavus (SQU21) and A. parasiticus (CBS921.7) were reported. The results showed that the oil of baobab significantly (p < 0.05) inhibited total aflatoxin secretions up to 47.2-95.7% for A. flavus and 42.7-93.3% for A. parasiticus, whereas aflatoxin B₁ showed inhibition of 28.1-89.7% and 25.9-80.2%, respectively. The mycelial dry weights of the Aspergillus strains were significantly (p < 0.05) reduced by the tested concentrations of A. digitata oil. This indicates the antifungal and inhibitory effects of baobab essential oil against the growth and aflatoxin production by the two strains of A. flavus (SQU21) and A. parasiticus (CBS921.7). Similar findings were reached by many authors using different oils from Nigella sativa (El-Nagerabi et al., 2012), cassia and bay (Attanda et al., 2007), Cymbopogon flexuosus (Kumar et al., 2009), Callistemon lanceolatus (Shukla et al., 2012), Zataria multiflora (Gandomi et al., 2009), and Ocimum gratissimum (Prakash et al., 2011).
3.3 Detoxification of Aflatoxins B₁ by Fruit Extract and Essential Oil of Baobab

Detoxifications with biological factors 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 ability of some herbal and medicinal plants as detoxifying agents was suggested by many researchers (Sandoskumar et al., 2007; El-Nagerabi et al., 2012). This includes Garlic (Allium sativum L. x) and onion (Allium cepa L.) roots extracts which cause 58.5% reduction in aflatoxin B₁. Seed extract of Trachyspermum ammi degraded 90% of aflatoxin G₁ by altering the ring structure of lactone (Velazhahan, Vijayanandraj, Vijayasamundeeswari, Parandidharan, Samiyappan, Iwamoto, Friebe, & Muthukrishnan, 2010). The presence of inactivation factors in T. ammi seed extract was responsible from 80% reduction of total aflatoxin content (Hajare, Haijare, & Sharma, 2006). In the present evaluation, the strains of the two selected Aspergillus species are aflatoxin-producers and secreting different level of aflatoxins. In the present study, we investigated the effect of the the highest concentrations of fruit pulp (7% w/v) and oil extract (5% v/v) of A. digitata on 780 ppb aflatoxin B₁ incubated at 25-29°C for 10 days. The results showed that the two extracts (7% fruit pulp 774 ppb; 5% oil 776 ppb) have no detoxification effect on pure aflatoxin B₁ comparison with the control (780 ppb). This suggests the non-detoxification properties of these extracts on aflatoxin B₁. Therefore, it is apparent that fruit and oil extracts of A. digitata had antifungal and inhibitory effect on aflatoxin secretion by Aspergillus strains (A. flavus SQU21 and A. parasiticus CBS921.7).

4. Conclusion

This paper describes the effects of fruit pulp extract and essential oil from the seeds of A. digitata (Baobab) on the growth and inhibition of aflatoxin production of A. flavus (SQU21) and A. parasiticus (CBS921.7). As far as we know, this is the first report on the biological activities of baobab on the fungal growth and aflatoxin secretion by aflatoxigenic fungi. The overall results demonstrate that both fruit extract and essential oil of this plant inhibited the mycelial dry weights and aflatoxins production by the two strains of Aspergillus species. The two extracts did not detoxify pure aflatoxin B₁. This indicates the antifungal activities and inhibitory effect of A. digitata extracts against moulds contamination. Therefore, baobab fruit and its essential oil can be suggested as plant additives and biopreservatives which enhance the nutritive value, quality, and protection against aflatoxin contamination as well as storage life. More phytochemical analysis is needed to identify the active chemical ingredients and testing their antimicrobial activities against different microorganisms and mycotoxins invasion. This will bring to the literature useful information which eventually promotes the quality of food and feed products and related agricultural and pharmaceutical industries.

![Graph](image-url)  
Figure 1a. Total aflatoxin production of A. flavus strain SQU21 and A. parasiticus strain CBS921.7 at different concentrations of A. digitata fruit extract (Identical numbers and letters indicate no significant difference, p < 0.05)
Figure 1b. Aflatoxin B₁ production of *A. flavus* strain SQU21 and *A. parasiticus* strain CBS921.7 at different concentrations of *A. digitata* fruit extract (Identical numbers and letters indicate no significant difference, *p* < 0.05)

Figure 1c. Mycelial dry weight of *A. flavus* strain SQU21 and *A. parasiticus* strain CBS921.7 at different concentrations of *A. digitata* fruit extract (Identical numbers and letters indicate no significant difference, *p* < 0.05)

Figure 2a. Total aflatoxin production of *A. flavus* strain SQU21 and *A. parasiticus* strain CBS921.7 at different concentrations of *A. digitata* oil extract (Identical numbers and letters indicate no significant difference, *p* < 0.05)
Figure 2b. Aflatoxin B₁ production of *A. flavus* strain SQU21 and *A. parasiticus* strain CBS921.7 at different concentrations of *A. digitata* oil extract (Identical numbers and letters indicate no significant difference, *p* < 0.05)

Figure 2c. Mycelial dry weight of *A. flavus* strain SQU21 and *A. parasiticus* strain CBS921.7 at different concentrations of *A. digitata* oil extract (Identical numbers and letters indicate no significant difference, *p* < 0.05)

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