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EFFECT OF *EPHEDRA ALATA* DECNE. ON LIPIDS METABOLISM OF *ASPERGILLUS FLAVUS* LINK

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Abstract

In *Aspergillus flavus* Link, the total lipid, sterols, neutral lipids, phospholipids and fatty acid content decreased significantly with the application of different concentrations of *Ephedra alata* Decne. extrtact. Gas chromatographic analysis revealed the presence of 12 fatty acids namely, (caprylic (C_8), capric (C_{10}), lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), palmitoleic ($C_{16:1}$), stearic (C_{18}), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), α linolenic ($C_{18:3}$), arachidic (C_{20}) and arachidonic ($C_{20:4}$) with total un-saturation per cent 69.78 in the cellular lipids of *A. flavus*. The use of *E. alata* extracts induced significant alteration in fatty acid profile towards increment saturation.

Introduction

Aspergillus flavus Link is widely distributed in soil, seeds, animal fodder, human food, and agricultural commodities (Alqarawi and Abd_Allah 2010). Aflatoxins are mutagenic and hepatocarcinogenic secondary metabolites produced by some fungi including *A. flavus* (Alqarawi and Abd_Allah 2010). Helal *et al*. (2007) reported the effects of *Cymbopogon citratus* L. essential oil on the growth, morphogenesis and aflatoxin production of *A. flavus*. Alqarawi *et al*. (2011) reported the production of aflatoxins by *A. flavus* on both maize grains and soybean seeds.

Safe and natural food, without chemicals is a demand by the modern society. In recent years, investigations were focused on developing alternative non-chemical strategies against aflatoxigenic seedbrone *A. flavus* (Alqarawi and Abd_Allah 2010, Alqarawi *et al*. 2011). Non-chemical approach to resisting Aflavotoxin of *A. flavus* has been done via several means using *Ephedra* plant (Reichling *et al*. 2009, Alqarawi *et al*. 2011). Our previous investigation reported the antifungal potential of *E. alata* against growth parameters and aflatoxins production of *A. flavus in vitro* and *in vivo* has also been investigated (Alqarawi *et al*. 2011). Antifungal resistance system in *A. flavus* has been expressed as number of defense-related mechanisms (Helal *et al*. 2007, Alqarawi *et al*. 2011). Lipids metabolism has been used as sensitive monitor for mold-plant interaction (Abd_Allah *et al*. 2006, Van der Meer-Janssen *et al*. 2010). However limited information is available regarding the compliance of cellular lipids in *A. flavus* facing natural antifungal compounds of plant origin.

The objective of the present research was to have better understanding of lipids metabolism in *A. flavus* as sensitive monitor for the antifungal mechanism of *E. alata*.

Materials and Methods

The plant samples (*E. alata*) were collected from King Khalid Centre (KKC) of Wildlife Research and Development at Thumama, Riyadh, SA, in 2010. The samples were air dried, powdered and extracted with aqueous ethanol (10:90, v/v) at 30°C for overnight and filtered through double layers of Whatman No. 1 filter paper after Alqarawi *et al*. (2011).

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Aspergillus flavus isolated from seed were used for the present experiment (Alqarawi *et al*. 2011). The growth of *A. flavus* was carried out at static state (28ºC ± 1) for 10 days using glucose-ammonium nitrate salt broth medium (Brain *et al*. 1961) the culture was supplemented with different concentrations (0.5, 1.0 and 2.0%, w/v) of range plant *E. alata* extracts (Alqarawi *et al*. 2011). Control flasks were used as references. At the end of incubation period, the culture broths were filtered through pre-weighted filter paper (Whatman No.1). The filter papers with mycelial growth washed carefully with distilled water followed by drying at 80^{o}C up to two successive weights were obtained. The net dry weight of mycelial growth determined and expressed as per cent of control flasks.

Lipid contents were extracted from the mycelia of *A. flavus* as described by Fölsh *et al*. (1957). Total lipids, neutral lipids and phospholipids were estimated according to Marsh and Weinstein (1966), Amenta (1964) and Rouser *et al*. (1970), respectively. Methanolysis was done according to Kates (1972) and methyl esters were analyzed according to Johnson and Stocks (1971). Standard fatty acids samples were used as reference. The statistical analysis was carried out according to Daniel (1987).

Results and Discussion

The extracts of *E. alata* at concentrations of 0.5, 1.0 and 2.0 (w/v) were effective against *A. flavus*. Significant decrease in mycelial growth by per cent of 33.5, 56.9 and 79.8, respectively compared with control was observed (Fig 1). Species belonging to *Ephedra* possess variable antifungal potential against many pathogen plants as well as seedbrone aflatoxigenic molds such

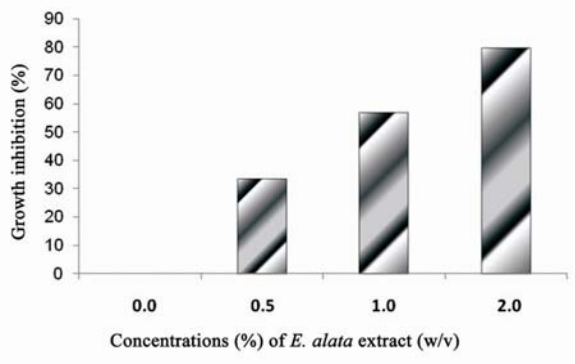

Fig. 1. Effect of different concentrations (Control, 0.5, 1.0, 2.0%) of *E. alata* extract (w/v) on growth inhibition (%) of *A. flavus*.

Table 1. Effect of different concentrations of *E. alata* extracts (w/v) on total lipid, total sterols, total neutral lipid and total phospholipid contents (mg/g dry weight) of *A. flavus*.

Treatments	Lipids fractions contents (mg/g dry weight) of *A. flavus*			
	TL	TS	TN	TP
Control	6.44	3.27	22.84	12.62
0.5 % (w/v)	5.05	3.07	17.12	10.17
1.0 % (w/v)	3.71	2.73	13.06	6.58
2.0 % (w/v)	1.84	2.16	8.04	4.46
LSD at: 0.5	0.61	0.52	2.45	1.00

TL = total lipid, TS = total sterol, TN = total neutral lipid, TP = total phospholipids.

Table 2. Effect of different concentrations of *E. alata* (w/v) on cellular fatty acids profile of *A. flavus*.

Treatments	Fatty acids profile of *A. flavus* (%)												
	Caprylic C8	Capric C10	Lauric C12	Myristic C14	Palmitic C16	Palmitoleic C16:1	Stearic C18	Oleic C18:1	Linoleic C18:2	α Linolenic C18:3	Arachidic C20	Arachidonic C20:4	Un-saturation %
Control	0.11	0.21	3.05	3.02	7.35	0.93	16.37	14.81	27.09	6.90	0.09	12.56	69.78
0.5% (w/v)	o.66	0.38	4.59	4.35	9.25	0.71	20.00	13.07	25.01	5.07	0.25	10.32	60.50
1.0% (w/v)	0.94	0.72	6.18	6.76	10.35	0.42	23.89	11.57	22.98	3.03	0.58	7.11	50.57
2.0% (w/v)	2.17	1.24	9.97	7.18	14.86	0.18	29.18	9.67	16.81	1.5	1.08	3.57	36.32
LSD at 0.5	0.68	0.34	0.71	1.14	1.12	0.13	1.21	1.25	2.37	0.99	0.19	1.54	

as *Aspergillus parasiticus* (Bagheri *et al*. 2009) and *Aspergillus flavus* (Alqarawi *et al*. 2011). Also, recent investigation revealed the antimicrobial potential of *Ephedra* against pathogenic bacteria (Cottiglia *et al*. 2005). The antifungal activity of *E. alata* has been attributed because of the presence of cis-3,4-methanoproline (Caveney *et al*. 2001), citronellol (Rosato *et al*. 2007) and heptadecane (Bagheri *et al*. 2009).

The *E. alata* extracts of caused significant decrease in contents of total lipids, total sterols, total neutral lipids and phospholipids of *A. flavus* as compared with those of control (Table 1). It has been established that lipid moieties are an important materials in biological membranes playing an essential role in their permeability (Georgopapadakou and Walsh 1996). Similar alteration has been recognized in *Trichoderma koningii* (El-Mougith 1999) stressed by different chemical fungicides. It was also reported that the incorporation of fungal lipid moieties strongly inhibited in response to antifungal compounds from plant origin (Helal *et al*. 2007) which caused an alterations in fungal cell membrane function and leakage of ions (Reichling *et al*. 2009).

Gas chromatographic analysis of cellular fatty acids (methyl ester) of *A. flavus* revealed the presence of 12 fatty acids, namely caprylic (C_8), capric (C_{10}), lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), palmitoleic ($C_{16:1}$), stearic (C_{18}), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), α linolenic ($C_{18:3}$), arachidic (C_{20}) and arachidonic ($C_{20:4}$) (Table 2). The saturated fatty acids were caprylic (C_8), capric (C_{10}), lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), stearic (C_{18}), arachidic (C_{20}) and un-saturated fatty acids were palmitoleic ($C_{16:1}$), Linoleic ($C_{18:2}$), α Linolenic ($C_{18:3}$), arachidonic ($C_{20:4}$). Present results corroborate those of Wilson *et al*. (2004), Helal *et al*. (2007) and Zain (2009) who reported that oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids were the most common unsaturated fatty acids in cellular lipids of *Aspergillus*. The present investigation provides that the different concentrations of *E. alata* extract caused significant decrease in total un-saturated fatty acids ($C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{20:4}$). The percent of inhibition of total un-saturated fatty acids was 13.3, 27.5 and 47.9, as compared with control (Table 2). Per cent of un-saturated fungal fatty acids has been reported as an important biochemical and physiological monitor for fungal development and adaptation (Wilson *et al*. 2004). The alteration in fatty acids composition has been related to mold resistance against the reverse biotic stress (Helal *et al*. 2007) to maintain the membrane fluidity of living cell (Davidse 1973). A decrease in membrane viscosity was antagonized by synthesis of saturated fatty acids (Rosas *et al*. 1980).

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