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EFFECT OF PROPOLIS ON GROWTH, AFLATOXINS PRODUCTION AND LIPID METABOLISM IN *ASPERGILLUS PARASITICUS* SPEAR

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Abstract

The mechanism of antifungal activities of propolis (Bee glue) was investigated for the growth, aflatoxins production, and lipids (total lipids, neutral lipids, phospholipids, and fatty acids) metabolism of *Aspergillus parasiticus*. The results of the present study indicated that propolis caused significant decrease in conidial production and conidial germination as well as mycelial growth (both radial and dry weight) of *A. parasiticus*. It was also found that aflatoxins production by *A. parasiticus* decreased significantly with 0.2 and 0.4 (g/100 ml) concentrations of propolis, however, 0.6 (g/100 ml) concentration caused complete inhibition of all aflatoxins production. The biochemical investigation of cellular total lipids, neutral lipids, and phospholipids of *A. parasiticus* suggested clear catabolic repression of lipids metabolism by propolis. Gas chromatographic analysis of cellular fatty acids indicated that propolis enhanced accumulation of saturated fatty acids suggesting resistance mechanism of fungal membrane via decreasing its fluidity and elasticity.

Introduction

Propolis [PR] (Bee glue) is a mixture of bee wax and resins collected by the honeybee (*Apis mellifera*) from parts of plant, buds, flower and exudates (Ghisalberti, 1979). Propolis has been used to seal holes, exclude draught, and protect the beehive against external invaders. The main function of propolis is to prevent the decomposition of organic matter within beehive by inhibiting microbial growth and activity (Quiroga *et al*., 2006). Many biological activities of propolis have been reported such as medicinal (Orsolic *et al*., 2003) antibacterial (Uzel *et al*., 2005) and antifungal (Ghaly *et al*., 1998; Kujumgiev *et al*., 1999; Khezri *et al*., 2006; Buchta *et al*., 2011). Generally, many biological active compounds have been identified in propolis such as polyphenols, phenolic aldehyde, sequiterpene quinines, coumarins, amino acids, steroids, and inorganic compounds (Kosalec *et al*., 2004; Uzel *et al*., 2005**;** Katircioglu & Mercan 2006). The biological activities of propolis vary depend up on geographical origin and bearing plants (Kujumgiev *et al*., 1999).

Aflatoxins are mutagenic and hepatocarcinogenic secondary metabolites produced by some strains of *Aspergillus parasiticus* (Abd_Allah & Hashem, 2006). *A. parasiticus* has been reported in many agricultural commodities (Abd_Allah & Hashem, 2006; Alqarawi & Abd_Allah, 2010; Elshafie *et al*., 2011) causing many agricultural and medicinal problems, hence it has been selected as an aflatoxigenic mold model in our study. The usage of systemic chemicals is primary method to control of *A. parasiticus*. Currently several chemical fungicides have been rejected and removed from the markets due to possible toxicological risks (Adaskaveg & Förster, 2010). Consequently, there is urgent need to develop non-chemical alternative strategies to control *A. parasiticus* and bioremediation of their mycotoxins (Abd_Allah & Ezzat, 2004; Helal *et al*., 2007; Alqarawi & Abd_Allah, 2010; Alqarawi *et al*., 2011).

It has been established that lipids moieties are an important materials in biological membranes (Hitchcock, 1975) playing an essential role in their permeability (Fritsche, 2006**;** Shaikh & Edidin**,** 2008). Signature of fatty acids was an objective tool for evaluating the growth and development of many fungi (Latge & Biévre, 1980; Lösel, 1989**;** Fakas *et al*., 2006**).** Also, lipids metabolism have been used as sensitive monitor for plant-mold interaction (Abd_Allah & Ezzat, 2004; Abd_Allah, 2005; Abd_Allah *et al*., 2006; Van der Meer-Janssen *et al*., 2009). In continuous connection, lipids metabolism of *A. flavus* has been used as sensetive monnitor for defense-related mechanism against biotic stress of antifungal from plant origin (Helal *et al*., 2007; Alqarawi & Abd_Allah, 2012).

The purposes of this study were investigating the antifungal mechanism of propolis collected from Saudi Arabia on growth, aflatoxins production and lipids metabolism of *A. parasiticus* as an aflatoxigenic mold model.

Materials and Methods

Microorganisms: *Aspergillus parasiticus* Spear. was isolated from camel fodder sample collected from camel farm near Riyadh city, Saudi Arabia. The identification of experimental mold was carried out according to Domsch *et al*., 1993). Aflatoxin-sensitive strain of *Bacillus brevis* was kindly provided by Dr. Gamal El-Didamony, Botany Department, Faculty of Science, Zagazig University, Egypt.

Preparation of PR extract: Propolis was collected from colonies at Abha city, Saudi Arabia and it was scrapped-off the top of frames and inner wall boxes of bee colonies. PR was extracted with aqueous:ethanol (10:90, v/v) as described by Ghaly *et al*., (1998). Based on preliminary experiment, three concentrations (0.2, 0.4 and 0.6; g/100ml) of PR were selected.

Growth characteristics of *A.* parasiticus: Czapek-Dox agar medium (Raper & Fennel, 1965) was used for the growth of experimental mold (*A. parasiticus*). Broth culture was used for measuring the dry weight of the test fungus using 100 ml medium in 250 ml capacity

Erlenmeyer flasks. Mycelial dry weight was estimated by filtration the broth mold culture after ten days of incubation at 28± ^{o}C in dark at static state, on Whatman no 1 filter paper. Mycelium growth was washed carefully with distilled water, dried at 105^{o}C up to two successive constant weights, then the mycelia dry weight was recorded. The test fungus was also grown in agar medium in 9.0cm diameter Petri-dishes for seven days incubated at 28± ^{o}C in dark and the radial growth was measured. Conidial production and their germination were studied according to Roberts & Selitrenikoff (1988).

Aflatoxins analysis: The extraction and clean-up of aflatoxins was carried out according to Dutton & Westlake (1985). Column chromatography study was carried out using silica gel G-60, mesh 0.02-0.2 (BDH Chemicals, Poole, UK) according to (Anonymous, 1970). The biological confirmatory test of aflatoxins was performed with the help of aflatoxins-sensitive strain of *Bacillus brevis* by following Madhyastha *et al*., (1994). The chemical confirmatory tests were done according to (Anonymous, 1970) using Toluene: Ethyl acetate: Formic acid (6: 3: 1, v/v/v) as mobile phase. Thin layer chromatography (TLC) plates (20X20, cm) coated with 20µ thick silica gel DG (Kieselgel-DG, Rieldel-De haen, Seelze-Hannover, Germany) was used for chromatographic separation of aflatoxins based on their Rf-value. The quantitative estimation of aflatoxins was spectrophotometrically determined by following Nabney & Nesbitt (1965). Standard aflatoxins (Sigma) were used as reference in both qualitative and quantitative estimation of aflatoxins.

Lipids analysis: Lipid contents were extracted from the mycelia growth of *A. parasiticus* using chloroform: methanol (2:1, v/v) according to Fölsh *et al*., (1957), 0.05% (w/v) of butylated hydroxytoluene (BHT; 2,6-di-tert-butyl-p-cresol) added to all solvents to prevent lipid peroxidation (Cachorro *et al*., 1993). Lipid extract was used for estimation of total lipids (Marsh & Weinstein, 1966); natural lipids (Amenta, 1964) and phospholipids (Rouser *et al*., 1970) contents. Phospholipid classes were separated by two-dimensional chromatography with $CHCl_3$-MeOH-[28% (w/v) NH_4OH] (13:5:1, v/v/v) for the first dimension and CHC3-Me2CO-MeOH-HOAc-H20 (6:8:2:2:1, v/v/v/v/v) for the second dimension (Rouser *et al*., 1970). Identification was made by comparison of Rf values with those of pure standards and by specific staining reaction (molybdenum reagent) according to Dittmer & Lester (1964). Spots were outlined with a pencil, scraped off of the plates, and total phospholipids were determined (Dittmer & Wells, 1969). Fatty acid methyl esters were prepared by methanolysis in H_2SO_4-MeOH (Kates, 1972). Esters were analyzed by gas liquid chromatography (GLC) (PerkinElmer Model 910, PerkinElmer, Shelton, CT, USA) equipped with a flame ionization detector (Johnson & Stocks, 1971). A dual-open recorder and a computing integrator (PerkinElmer Model M1) were attached to GLC for recording. The separation and quantization of peak fatty acid methyl esters were identified by comparing their retention times with those of an authentic methyl ester standard (Sigma Co., St. Louis, MO, USA).

Statistical analysis: All experiments were repeated at least three times. The data were statistically analyzed using analysis of variance for a completely randomized design (Daniel, 1987).

Results and Discussion

Both conidial production and conidial germination of the test fungus were increasingly decreased with the increase of concentration of PR in the medium in directly proportionally (Table 1). The decrease in conidial production and their germination recorded in the present study is similar with the reports of Ghaly et al., (1998). They have reported the decrease in germination of 2 isolates of *Aspergillus flavus* by PR. The results presented in Table 2 indicated that PR caused significant decrease in both radial growth and mycelia dry weight of *A. parasiticus***.** Similar antifungal activities of PR on growth of different fungi such as Candida albicans (Afrouzan *et al*., 2012**);** *Aspergillus parasiticus* (Khezri *et al*., 2006) and *Trichophyton mentagrophytes* (Buchta *et al*., 2011**)** have been reported**.** The phenols (Lisá *et al*., 1989) and flavonoids (Cushnie & Lamb, 2005) has been considered the main carriers of antifungal properties of PR. The antifungal mechanism of PR is still not complete clarity. In this regard, Takaisi-Kikuni & Schilcher (1994) reported that PR caused an inhibition in cell division hence suggested that PR might inhibit DNA replication.

Table 1. Effect of different concentrations of propolis (w/v) on number of germinated conidia (out 100 conidia) and conidial production (conidia mm^{2}X10^4) of *A. parasiticus*.

Concentration of propolis (g/100 ml)	Number of germinated conidia (out 100 conidia in number)	Conidial production (conidia mm^{2}X10^4)
Control	100	10.621
0.2 % (w/v)	62.37	4.275
0.4 % (w/v)	31.87	1.003
0.6 % (w/v)	10.35	0.246
LSD at: 0.05	6.21	0.397

Table 2. Effect of different concentrations of propolis (w/v) on radial growth (cm/plate) and mycelial growth (g/100 ml culture medium) of *A. parasiticus*.

Concentration of propolis (g/100 ml)	Radial growth (cm/plate)	Mycelial growth (g/100 ml culture medium)
Control	9.00	2.7531
0.2 % (w/v)	6.31	1.9243
0.4 % (w/v)	4.01	0.7251
0.6 % (w/v)	1.75	0.3042
LSD at: 0.05	0.987	0.124

The chromatographic analysis (based on Rf-value and the developed color) followed by chemical confirmatory tests demonstrated the presence of aflatoxins B_1, B_2 and G_1 in the culture filtrates of *A. parasiticus* (Table 3). The production of different aflatoxins by *A. parasiticus* was reported by many investigators (Klich, 2007). The qualitative and quantitative productions of aflatoxins by A. parasiticus mainly depend upon on its genetic characters (Kale *et al*., 2007); environmental surrounding conditions (Klich, 2007) and the nutritional composition of substrate (Wilkinson *et al*., 2007). In our results (Table 4), the lower concentrations (0.2 % and 0.4 %) of propolis showed significant deceased in aflatoxins (B_1, B_2, G_1 & total) production, but higher cons. (0.6 %) showed complete inhibition of the production of all types of aflatoxins. Our results corroborated those of Ghaly *et al*., (1998) and Khezri *et al*., (2006) who reported the inhibitory effect of PR on mycotoxins production by *A. flavus* and *A. parasiticus*, respectively. The inhibitory effect of PR on aflatoxins production attributes mainly on the presence of flavonoids, phenolic acid, benzoic acid and their esters (Park *et al*., 1997; Marcucci *et al*., 2001; Kosalec *et al*., 2004). The mechanism of PR activity depends on synergism among flavonoids, phenolic acids in PR and their interference with both biosynthesis of aflatoxins (direct effect) and mold growth (indirect effect). The kinetic production of total aflatoxins increased gradually with increasing PR concentrations (Table 4) indicating that the response of fungal (*A. parasiticus*) growth was more sensitive to PR concentrations than aflatoxins production. On the other hand, a genetic correlation between conidial production and secondary metabolites such as aflatoxins production has been demonstrated (Chang *et al*., 2004; Duran *et al*., 2007).

Table 3. Detection and chemical confirmatory tests of aflatoxins produced by *A. parasiticus*.

Concentration of propolis (g/100 ml)	R_f-value developed color under UV (366nm) light before p-anisaldehyde reagent	Color developed color under UV (366nm) light before p-anisaldehyde reagent	Developed color after chemical confirmation after p-anisaldehyde reagent	
			Day light	UV (366nm) light
B_1	11.47	Blue	Green	Orange
B_2	31.60	Green	Blue	Green
G_1	19.58	Blue	ND	Pale pink-orange

ND= Not detected under the experimental conditions.

Table 4. Effect of different concentrations of propolis (w/v) on aflatoxins (B_1, B_2; G_1) production (µg/100 ml culture medium) by *A. parasiticus*.

Concentration of propolis (g/100 ml)	Aflatoxins production (µg/100 ml culture medium)				
	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Total aflatoxins (B_1+B_2+G_1)	*Kinetic production of total aflatoxins
Control	297.2	143.5	107.6	548.3	199.15
0.2 % (w/v)	268.4	87.5	67.1	423.0	219.82
0.4 % (w/v)	143.7	37.2	14.3	195.2	269.20
0.6 % (w/v)	ND	ND	ND	ND	ND
LSD at: 0.05	23.7	13.7	8.4	47.6	

ND= Not detected under the experimental conditions. *: Kinetic production of total aflatoxins= total aflatoxins/mycelial growth.

The PR caused significant decreases in total lipids content of *A. parasiticus*, and such decreases was directly proportional with concentrations (Table 5). Similar inhibition in total lipids content was reported in Chaetomium globsum (Khashaba, 1995) and Trichoderma koningii (El-Mougith, 1999). In the same way, it was reported that natural antifungal from Cymbopogon citrates L. (Helal *et al*., 2007) and Ephedra alata (Alqarawi **&** Abd_Allah, 2012) were caused significant decrease in lipids content of Aspergillus flavus. Lipids degradation have been often associated with decline phase of mold growth (Shapira *et al*., 1984) due to inhibition of tricarboxylic acid and glyoxylate cycles (Brennan **&** Lösel, 1978) which directly influence growth and morphogenesis in fungi (Kritzman, 1976; Shapira *et al*., 1984). It was also found that PR caused an increase in DG, SE and FAA associated with significant decrease in TG and S as compared with those of *A. parasiticus*

control (Table 5). This general pattern of neutral lipids turnover has been reported in many fungi as resistant mechanism against chemical fungicides (Khashaba, 1995; El-Mougith, 1999**;** Fakas *et al*., 2006) may serve to overcome the harmful effect of antifungal on membrane permeability (Hernádez-Lauzardo *et al*., 2011). In another connection, the degradation of TG into DG and inhibition of S biosynthesis consequently, increase SE in plant cells has been reported as resistance mechanism against biotic stress of plant pathogen (Abd Allah *et al*., 2006) and disease-inducing agent, fusaric acid (Abd_Allah **&** Ezzat, 2004).

Table 5. Effect of different concentrations of propolis (w/v) on total lipids content (% of dry mycelial weight) and neutral lipids fractions (mg/g dry weight) of *A. parasiticus*.

Concentration of propolis (g/100 ml)	Total lipids content (% of dry mycelial weight)	Neutral lipids fractions (mg/g dry weight)				
		TG	DG	S	SE	FAA
Control	0.7354	28.3	13.7	18.9	8.7	6.4
0.2 % (w/v)	0.5837	25.4	15.9	16.1	10.3	8.1
0.4 % (w/v)	0.3084	20.7	21.3	11.7	12.1	9.3
0.6 % (w/v)	0.1275	16.3	27.8	8.72	14.9	10.2
LSD at: 0.05	0.0783	2.18	1.37	2.14	0.75	0.34

TG, triacylglycerol; DG, diacylglycerol; S, sterol; SE, sterol ester; FAA, non-esteried fatty acids.

In phospholipids investigation, PR decreases all phospholipids fractions (PC, PE, PG, PI, PS) except PA (Table 6) indicating a blocking in the pathway of other phospholipids fractions from PA as reported by Kates **&** Marshall (1975). Such decrease in phospholipids fractions reported in our data due to PR agrees with the inhibitory effect of chemical antifungal on other fungi (Radzuhn **&** Lyr, 1984; Khashaba, 1995; El-Mougith, 1999). The antifungal compounds from plant origin caused similar effect on phospholipids of fungal cell wall (Helal *et al*., 2007; Alqarawi **&** Abd_Allah, 2012). It has been reported that, blocking of phospholipids biosynthesis by antifungal which induce yeast-like growth of filamentous fungi (Radzuhn **&** Lyr, 1984; Khashaba, 1995). In the same connection, chitin synthases enzyme (which responsible for chitin synthesis in fungal cell wall) are integral membrane proteins and require phospholipids for normal functioning (Deshpande *et al*., 1997).

Table 6. Effect of different concentrations of propolis (w/v) on phospholipids fractions (mg/g dry weight) of *A. parasiticus*.

Concentration of propolis (g/100 ml)	Phospholipids lipids fractions (mg/g dry weight)					
	PC	PE	PG	PI	PS	PA
Control	29.3	41.8	6.2	18.6	10.4	32.1
0.2 % (w/v)	24.6	36.8	4.9	12.9	7.3	38.2
0.4 % (w/v)	21.0	31.4	3.2	10.3	6.1	41.7
0.6 % (w/v)	16.3	27.0	1.8	7.5	4.7	43.9
LSD at: 0.05	2.37	3.41	0.71	1.34	0.82	2.12

PC, phosphatidyl choline; PE, phosphatidyl ethanol; PG, phosphatidyl glycerol; PS, phosphatidyl serine; PI, phosphatidyl inositol; PA, phosphatidic acid.

Gas chromatographic analysis of cellular fatty acids of *A***.** parasiticus control revealed the presence of 14 fatty acids namely caprylic (C8), capric (C10), lauric (C12), myristic (C14), palmitic (C16), palmitoleic (C16:1), margaric (C17), stearic (C18), oleic (C18:1), linoleic (C18:2), α linolenic (C18:3), arachidic (C20), cis-11 eicosenoic (C20:1) and arachidonic (C20:4) with un-saturation percent 59.68 (Table 7). Such fatty acids have been reported as common fatty acids in different isolates of *A. parasiticus* (Wilson *et al*., 2004; Chang *et al*., 2004). Also our results indicated appearance of caprylic [C8] fatty acid and increase the cellular content of all saturated fatty acids **(**capric [C10], lauric [C12], myristic [C14], palmitic [C16], margaric [C17], stearic [C18], arachidic [C20]**)** with clear decrease in total unsaturation percent as compared with control one (Table 7). Similar results have been reported by other antifungal compounds from synthetic chemical origin (Khashaba, 1995; El-Mougith, 1999) and natural plant origin (Helal *et al*., 2007; Alqarawi **&** Abd_Allah, 2012). Moreover, the data (Table 7) indicated clearly that all concentrations of PR caused complete disappearance and inhibition of three unsaturated fatty acids namely palmitoleic (C16:1), cis-11 eicosenoic (C20:1) and arachidonic (C20:4). Such general pattern of fatty acids transformation recorded here in our data were supported by report of O_2 generation developed (Kapich *et al*., 2011) as resistance mechanism developed against un-favorable conditions might stresses the biological cells (Gill & Tuteja, 2010). The accumulation of saturated fatty acids in the biological cell could possibly indicate that there were decrease in fluidity and elasticity of cell membrane consequently unbalance of its selective permeability (Starkov *et al*., 1994; Furuno *et al*., 2001; Fritsche, 2006). In continuous connection, it was reported that accumulation of polysaturated fatty acids in the biological cell has been correlated with high rate of membrane resistance compared to that display low incidence (Fritsche, 2006; Shaikh & Ediden, 2008). The changes in fatty acids composition has been related to antifungal potential of flavonoids, phenolic acid, benzoic acid and their esters (Park *et al*., 1997; Marcucci *et al*., 2001; Kosalec *et al*., 2004) which have been reported as predominant biologically-active compounds in PR (Kosalec *et al*., 2004; Uzel *et al*., 2005; Katircioglu & Mercan 2006).

Table 7. Effect of different concentrations of propolis (w/v) on cellular fatty acids profile of *A. parasiticus*.

Concentration of propolis (g/100 ml)	Fatty acids profile of *A. parasiticus* (%)														
	Caprylic C8	Capric C10	Lauric C12	Myristic C14	Palmitic C16	Palmitoleic C16:1	Margaric C:17	Stearic C18	Oleic C18:1	Linoleic C18:2	α Linolenic C18:3	Arachidic C20	Cis-11 Eicosenoic C20:1	Arachidonic C20:4	Un-saturation %
Control	0.00	1.58	4.74	2.34	8.35	0.97	1.04	21.80	17.43	20.72	15.65	0.47	0.76	4.15	59.68
0.2 % (w/v)	1.04	3.17	7.43	4.15	10.58	0.00	2.01	26.04	13.27	17.39	13.61	1.31	0.00	0.00	44.27
0.4 % (w/v)	2.65	5.25	5.64	6.46	12.74	0.00	4.01	28.13	10.24	12.82	8.58	3.48	0.00	0.00	31.64
0.6 % (w/v)	3.47	7.02	6.34	9.02	13.98	0.00	5.27	30.43	7.25	7.32	4.65	5.25	0.00	0.00	19.22

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References

Abd_Allah, E.F. 2005. Effect of a *Bacillus subtilis* isolate on southern blight (*Sclerotium rolfsii*) and lipid composition of peanut seeds. *Phytoparasitica*, 32: 460-466.

Abd_Allah, E.F. and S.M. Ezzat. 2004. Role of lipid metabolism through bioremediation of fusaric acid in germinating peanut seedlings. *Phytoparasitica*, 32: 38-42.

Abd_Allah, E.F. and Hashem Abeer. 2006. Seed mycoflora of *Lens esculenta* and their biocontrol by chitosan. *Phytoparasitica*, 34: 213-218.

Abd_Allah, E.F.; Abeer Hashem and S.M. Ezzat. 2006. Lipid metabolism in tomato and bean as a sensitive monitor for biocontrol of wilt diseases. *Phytoparasitica*, 34: 516-522.

Adaskaveg, J. E. and H. Förster. 2010. New Developments in Postharvest Fungicide Registrations for Edible Horticultural Crops and Use Strategies in the United States. In: *Postharvest Pathology* (Eds.): D. Prusky, M. Lodovica, Gullino Springer, New York, pp.211.

Afrouzan, H., C. Amirinia, S.A. Mirhadi, A. Ebadollahi, N. Vaseji and G. Tahmasbi. 2012. Evaluation of antimicrobial activity of propolis and nanopropolis against *Staphylococcus aureus* and *Candida albicans*. *African J. Microbiol. Res.*, 6: 421-425.

Alqarawi, A.A. and E.F. Abd_Allah. 2010. Maintenance of *Ephedra alata* seed viability via storage containers. *Amer. J. Plant Sci.*, 1: 138-146.

Alqarawi, A.A. and E.F. Abd_Allah. 2012. Effect of *Ephedra alata* on lipids metabolism of *Aspergillus flavus*. *Bangladesh J. Bot.,* (Accepted).

Alqarawi, A.A., E.F. Abd_Allah and Hashem Abeer. 2011. *Ephedra alata* as biologically-based strategy inhibit aflatoxigenic seedborne mold. *African J. Microbiol. Res.*, 5: 2297-2303.

Amenta, J.S. 1964. A rapid method for quantifcation of lipids separated by thin layer chromatography. *J. Lipid Res.*, 5: 270-272.

Anonymous. 1970. AOAC (Association of Official Analytical Chemists). Official Methods of Analysis (11th ed.), Section 26.019 (a), 429. AOAC, Washington, D.C.

Brennan, J. and D.M. Lösel. 1978. Physiology of fungal lipids: selected topics. *Advances in Microbial Physiology*, 17: 48-164.

Buchta, V., J. Černý and V. Opletalová. 2011. In vitro antifungal activity of propolis samples of Czech and Slovak origin. *Central European J. Biology*, 6: 160-166.

Cachorro, P., A. Ortiz and A. Cerda. 1993. Effect of saline stress and calcium on lipid composition in bean roots. *Phytochemistry*, 32: 1131-1136.

Chang, P.K., R.A. Wilson, N.P. Keller and T.E. Cleveland. 2004. Deletion of the Δ^{12}-oleic acid desaturase gene of a non-aflatoxigenic *Aspergillus parasiticus* field isolate affects conidiation and sclerotial development. *J. Appl. Microbiol*., 97: 1178-1184.

Cushnie, T.P.T. and A.J. Lamb 2005. Antimicrobial. activity of flavonoids. *International J. Antimicrobial Agents*, 26: 343-356.

Daniel, W.W. 1987. Biostatistics: A foundation for Analysis in the Health Science. 4th ed., John Wiley and Sons, New York, NY. pp. 292-293.

Deshpande, M.V., R. O'Donnell and G.W. Gooday. 1997. Regulation of chitin synthase activity in the dimorphic fungus *Benjaminiella poitrasii* by external osmotic pressure. *FEMS Microbiol. Lett.*, 152: 327-332.

Dittmer, J.C. and R.L. Lester. 1964. A simple, specific spray for the detection of phospholipids on thin layer chromatograms. *J. Lipid Res.*, 5: 126-127.

Dittmer, J.C. and M.A. Wells. 1969. Quantitative and qualitative analysis of lipid and lipid components. *Methods Enzymol.*, 14: 482-530.

Domsch, K.H., W. Gams and T.H. Anderson. 1993. Compendium of Soil Fungi. Academic Press, London, pp. 860.

Duran, R.M., J.W. Cary and A.M. Calvo. 2007. Production of cyclopiazonic acid, aflatrem, and aflatoxin by *Aspergillus flavus* is regulated by veA, a gene necessary for sclerotial formation. *Appl. Microbiol. Biotech.*, 73:1158-1168.

Dutton, M.F. and K. Westlake. 1985. Occurrence of mycotoxins in cereals and animal feedstuffs in Natal, South Africa. *J. Assoc. Off. Anal. Chem.*, 86: 839-842.

El-Mougith, A.A. 1999. Effect of benomyl on the growth and lipid composition of *Trichoderma koningii*. *Folia Microbiol*., 44: 41-44.

Elshafie, S.Z.B., A. ElMubarak, S.A.F. El-Nagerabi and A.E. Elshafie. 2011. Aflatoxin B_1 contamination of traditionally processed peanuts butter for human consumption in Sudan. *Mycopathologia*, 171: 435-439.

Fakas, S., S. Papanikolaou, M. Galiotou-Panayotou, M. Komaitis and G. Aggelis. 2006. Lipids of *Cunninghamella echinulata* with emphasis to γ-linolenic acid distribution among lipid classes. *Appl. Microbiol. Biotechnol.*, 73: 676-683.

Fölsh, J., M. Lees and G.H. Sloane-Stanley. 1957. A simple method for the isolation and puri cation of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497-509.

Fritsche, K. 2006. Fatty acids as modulators of the immune response. *Annu. Rev. Nutr.*, 26: 45-73.

Furuno, T., T. Kanno, K. Arita, M. Asami, T. Utsumi, Y. Doi, M. Inoue and K. Utsumi. 2001. Roles of long chain fatty acids and carnitine in mitochondrial membrane permeability transition. *Biochem. Pharmacol.*, 62: 1037-1046.

Ghaly, M.F., S.M. Ezzat and M.M. Sarhan. 1998. Use of propolis and ultra- griseofulvin to inhibit aflatoxigenic fungi. *Folia Microbiol.*, 43:156-160.

Ghisalberti, E.L. 1979. Propolis: A review. *Bee World*, 60: 59-84.

Gill, S.S. and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.*, 48: 909-930.

Helal, G.A., M.M. Sarhan, A.N.K. Abu Shahla and E.K Abou El-Khair. 2007. Effects of *Cymbopogon citratus* L. essential oil on the growth, morphogenesis and aflatoxin production of *Aspergillus flavus* ML2- strain. *J. Basic Microbiol.*, 47: 5-15.

Hernádez-Lauzardo, A.N., J. Vega-Pérez, M. G. V. Valle, N. S. Sánchez, A. Peña and G. Guerra-Sánchez. 2011. Changes in the functionality of plasma membrane of *Rhizopus stolonifer* by addition of chitosan. *J. Phytopathol.*, 159: 563-568.

Hitchcock, C. 1975. Structure and distribution of plant acyl lipids. In*: Recent Advances in the Chemistry and Biochemistry of Plant Lipids*. (Eds.): T. Galliard, E.I. Mercer, Academic Press, New York, NY. pp. 1-19.

Johnson, A. and R. Stocks. 1971. Gas-liquid chromatography of lipids. In: Biochemistry and Methodology of Lipids. (Eds.): A. Johnson, J. Davenport. Wiley Interscience, New York, NY.

Kale, S.P., J.W. Cary, N. Hollis, J.R. Wilkinson, D. Bhatnagar, J. Yu, T.E. Cleveland and J.W. Bennett. 2007. Analysis of aflatoxin regulatory factors in serial transfer-induced non-aflatoxigenic *Aspergillus parasiticus*. *Food Addit. Contam.*, 24: 1061-1069.

Kapich, A.N., T.V. Korneichik, K.E. Hammel and A. Hatakka. 2011. Comparative evaluation of manganese peroxidase and Mn (III) initiated peroxidation of C18 unsaturated fatty acids by different methods. *Enzyme and Microbial. Technol.*, 49: 25-29.

Kates, M. 1972. Techniques of lipidology. In: *Laboratory Techniques in Biochemistry and Molecular Biology*. (Eds.): T.W. Work, E. Work. North-Holland Publishing Co., Amsterdam, the Netherlands.

Kates, M. and M.O. Marshall. 1975. Biosynthesis of phosphoglycerides in plants. In: *Recent Advances in the Chemistry and Biochemistry of Plant Lipids*. (Eds.): T. Galliard, E. I. Mercer. Academic Press Inc. (London) Ltd., UK. pp. 115-159.

Katircioglu, H. and N. Mercan. 2006. Antimicrobial activity and chemical compositions of Turkish propolis from different region. *African J. Biotechnol.*, 5: 1151-1153.

Khashaba, H.E. 1995. Physiological Studies on Some Biologically Acitive Fungi isolated from the Soil of The United Arab Emirates. Ph.D. thesis, Zagazig University, Egypt.

Khezri, M., S. Rostami, R. S. Riseh and A. Alizadeh. 2006. Effect of propolis and clotrimazole on controlling aflatoxin in pistachio (*Pistacia vera* L.). *International J. of Agriculture & Biology*, 5: 606-608.

Klich, M.A. 2007. Environmental and developmental factors influencing aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*. *Mycoscience*, 48: 71-80.

Kosalec, I., M. Bakmaz, S. Pepeejnjak and S.V. Kneevi. 2004. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta Pharm.*, 54: 65-72.

Kritzman, G., Y. Okon, Y. Henis and I. Chet. 1976. Metabolism of L-threonine and its relationship to sclerotium formation in *Sclerotium rolfsii*. *J. Gen. Microbiol.*, 95: 78-86.

Kujumgiev, A., I. Tsvetkova, Yu. Serkedjieva, V. Bankova, R. Christov and S. Popov. 1999. Antibacterial, antifungal and antiviral activity of propolis from different geographic origin. *J. Ethnopharmacology*, 64: 235-240.

Latge, J. and C. De Biévre. 1980. Lipid composition of *Entomophthora obscura* Hall & Dunn. *J. Gen. Microbiol.*, 121: 151-158.

Lisá, M., I. Leifertová and J. Baloun. 1989. Ein Betrag zur fungistatischen Wirkung von Propolis (A contribution to the antifungal effect of propolis). *Folia Pharm.*, 13: 29-44, (in German).

Lösel, D.M. 1989. Functions of lipids: specialized roles in fungi and algae. In: *Microbial lipids*. (Eds.): C. Ratledge, SG. Wilkinson. Academic Press Inc. (London) Ltd., UK. pp 367-438.

Madhyastha, M.S., R.R. Marquardi, A. Masi, J. Borsa and A.A. Froshlich. 1994. Comparison of toxicity of different mycotoxins to several species of bacteria and yeast: Use of *Bacillus brevis* in a disc diffusion assay. *J. Food Protect.*, 57: 48-53.

Marcucci, M.C., F. Ferreres, C. García-Viguera, V.S. Bankova, S.L. De Castro, A.P. Dantas, P.H.M. Valente and N. Paulino. 2001. Phenolic compounds from Brazilian propolis with pharmacological activities. *J. Ethnopharmacol.*, 74: 105-112.

Marsh, J.B. and D.B. Weinstein. 1966. Simple charring method for determination of lipids. *J. Lipid Res.*, **7**: 574-576.

Nabney, J. and B.F. Nesbitt. 1965. A spectophotometric method for determining the aflatoxins. *Analyst*, 90: 155-160.

Orsolic, N., A.H. Knezevic, L. Sver, S. Terzic, B.K. Heckenberger and I. Basic. 2003. Influence of honey bee products on transplantable murine tumours. *Vet. Comp. Oncology*, 1: 216-226.

Park, Y.K., M.K. Koo, M. Ikegaki and J.L. Contado. 1997. Comparison of the flavonoid aglycone contents of *Apis mellifera* propolis from various regions of Brazil. *Arq. Biol. Tecnol.*, 40: 97-106.

Quiroga, E.N., D.A. Sampietro, J.R. Soberon, M.A. Sgariglia and M.A. Vattuone. 2006. Propolis from the northwest of Argentina as a source of antifungal principles. *J. Appl. Microbiol.*, 101: 103-110.

Radzuhn, B. and H. Lyr. 1984. On the mode of action of the fungicide etridiazole. *Pestic. Biochem. Physiol.*, 22: 14-23.

Raper, K.B. and D.I. Fennell. 1965. *The Genus Aspergillus*, pp 686 Baltimore: Williams & Wilkins Company.

Roberts, W.K. and C.P. Selitrenikoff. 1988. Plant cell and bacterial chitinase differ in antifungal activity. *J. Gen. Microbiol.*, 134: 169-176.

Rouser, G., S. Fleischer and A. Yamamoto. 1970. Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids and phosphorus analysis of spots. *Lipids*, 5: 494-496.

Shaikh, S.R. and M. Edidin. 2008. Polyunsaturated fatty acids and membrane organization: elucidating mechanisms to balance immunotherapy and susceptibility to infection. *Chem. Phys. Lipids*, 153: 24-33.

Shapira, R., Y. Henis, D. Sklan and I. Chet. 1984. Changes in fatty acids during morphogenesis in *Sclerotium roljsii***.** *J. Gen. Microbiol.*, 130: 1183-1191.

Starkov, A.A., O.V. Markova, E.N. Mokhova, E. Arrigoni-Martelli and Bobyleva V.A. 1994. Fatty acid-induced Ca^{+2} dependent uncoupling and activation of external pathway of NADH oxidation are coupled to cyclosporine A-sensitive mitochondrial permeability transition. *Biochem. Mol. Biol. Int.*, 32:1147-1155.

Takaisi-Kikuni, N.B. and H. Schilcher. 1994. Electron microscopy and microcalorimetric investigations of the possible mechanism of the antibacterial action of a defined propolis provenance. *Planta Medica.*, 60: 222-227.

Uzel, A., K. Sorkun, Ö. Önçag, D. Çogŭlu, Ö. Gençay and B. Salih. 2005. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. *Microbiol. Res.*, 160: 189-195.

Van der Meer-Janssen, Y.P.M., J. van Galen, J.J. Batenburg and J.B. Helms. 2009. Lipids in host-pathogen interactions: Pathogens exploit the complexity of the host cell lipidome. *Prog. Lipid Res.*, 49: 1-26.

Wilkinson, J.R., J. Yu, J.M. Bland, W.C. Nierman, D. Bhatnagar and T.E. Cleveland. 2007. Amino acid supplementation reveals differential regulation of aflatoxin biosynthesis in *Aspergillus flavus* NRRL 3357 and *Aspergillus parasiticus* SRRC 143. *Appl. Microbiol. Biotechnol.*, 74: 1308-1319.

Wilson, R.A., A.M. Calvo, P. Chang and N.P. Keller. 2004. Characterization of the *Aspergillus parasiticus* Δ^{12}-desaturase gene: a role for lipid metabolism in the Aspergillus-seed interaction. *Microbiology*, 150: 2881-2888.

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