Journal of Medicinal Plants Research Vol. 5(17), pp. 4231-4238, 9 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Evaluation of some plant extracts for their antifungal and antiaflatoxigenic activities

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Accepted 17 May, 2011

Antifungal and antiaflatoxigenic activities of five methanolic plant extracts were evaluated against the toxigenic *Aspergillus flavus* isolate. Extract of *Punica granatum* showed the highest antifungal and antiaflatoxigenic activities followed by *Zingiber officinalis*. *P. granatum* extract absolutely arrested aflatoxin B_1 production at 5 mg/ml and inhibited 100% the mycelial growth of *A. flavus* at 10 mg/ml. *Zingiber officinalis* and *Olea europaea* extracts showed a moderate antifungal activities and exhibited a significant antiaflatoxigenic efficiency as they completely inhibited aflatoxin B_1 production at 15 mg/ml. *Lantana camara* extract showed a weak antifungal activities while no effect was detected with *Allium sativum* L. The analysis of plant extracts by GC/MS showed that *P. granatum* extract was mainly composed by ellagic acid (37.01%), pedunculagin (6.40%), punicalugin (5.64%) and polyphenol as lumicolchicine (4.68%) while components of *Z. officinalis* were gingerol (46.85%), cedrene (8.39%), zingiberene (7.40%) and α-curcumene (7.32%) respectively. The findings obtained from this study may contribute to development of potentially effective and environmentally safer alternative fungicides to protect the spoilage of wheat grains from the toxigenic *A. flavus*.

Key words: Aflatoxins, wheat, antiaflatoxigenic, methanolic extract, *Punica granatum*, *Zingiber officinalis, Allium sativum.*

INTRODUCTION

Aflatoxins comprise a group of chemically diverse compounds originating from secondary metabolism of filamentous fungi especially *Aspergilllus flavus* and *Aspergilllus parasiticus*. Aflatoxins are stable under normal food processing conditions and can therefore be present not only in food and feed but also in processed products and threating both human and animal health as they are known to be the most potent carcinogens (Nizam and Oguz, 2003; Omidbeygi et al., 2007; Sidhu et al., 2009). These toxins have been incriminated as the cause of high mortality in livestock and some cases of death in human being (Park et al., 2004; Egal et al., 2005). Among all classes of aflatoxins, aflatoxin B_1 (AFB_1) is known to be the most significant in terms of animal and human health risk (Coulombe, 1993). Thus, foods contaminated with these toxigenic fungi and presence of aflatoxins is a major concern which has received worldwide attention due to their deleterious effect on human and animal health as well as their importance in international food trade (Williams et al., 2004; Soubra et al., 2009; Riba et al., 2010). Currently, there is a strong debate about the safety aspects of fungicides in use since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity (Prabhu and Urs, 1998). For these reasons, the use of numerous plant extracts, spices and their constituents may provide an alternative way to prevent fungal growth and mycotoxins formation (Vagi et al., 2005; Kumar et al., 2007; Lee et al., 2001, 2007). Many spices and some plant extracts with their essential oils have proven to be efficient antifungal against toxigenic moulds (Shahi et al., 2003; Guynot et al., 2005; Dikbas et al., 2008). Rasooli et al. (2008) and Kumar et al. (2008) demonstrated antifungal properties of twenty plant extracts including *Zingiber officinalis, Euclayptus citriodora* and *Lantana camara* used to suppress toxigenic and food borne moulds. Nguefack et al. (2004)

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Table 1. The ethnobotanical data, plant parts employed and the extract percentage yield of the selected plant species.

Plant species	Family	Common name	Plant part used[@]	Extract yield (%)
Allium sativum L	Alliaceae	Garlic	R	18.47
Lantanta camara	Verbenaceae	Lantanas	L	7.68
Olea europaea	Oleaceae	Olive	L	13.94
Punica granatum	Lythraceae	Pomegranate	F.S	18.56
Zingiber officinalis	Zingiberaceae	Ginger	R	4.20

[@]Plant parts used: L, leaves; R, rhizome; F.S, fruit shell.

and Bluma et al. (2008) evaluated 41 plant extracts and their essential oils on *A. flavus* and they reported that all essential oils could inhibit the growth of *A. flavus* while extract of *Allium sativum* has no effect on the toxigenic fungus. In addition, many species and herb such as basil, cinnamon, marigold, arak and spearmint (Ahmed et al., 2008; Soliman and Badeaa, 2002), garlic and onion (Benkeblia, 2004), ginger (Kumar et al., 2007; Pinto et al., 2006), cassia and sweat basil (Atand et al., 2007) have been reported to inhibit toxigenic and food borne moulds. In the present study, the effect of five kinds of plant extracts including *A. sativum*, *L. camara, Olea europaea*, *Punica granatum*, and *Z. officinalis*, on growth and aflatoxins production of *A. flavus* were evaluated.

MATERIALS AND METHODS

Fungal isolation

Wheat seeds were disinfected using 2.5% sodium hypochlorite for three minutes, rinsed three times in sterile distilled water and dried between layers of sterile filter paper (Whatmann, No. 1). Ten groups of wheat seeds, each with five seeds were plated out on 15 ml of the PDA medium (5 seeds /plate) and incubated at 25°C for 5 days. Subculturing was repeated several times for mycelial tips to obtain pure cultures which were preserved on potato dextrose agar slant till identified.

Fungal strain

The toxigenic isolate *A. flavus* was isolated from seeds of *Triticum* spp. and identified on the basis of colony and morphological characteristics (Raper and Fennell, 1977). The toxigenicity of the isolates was determined using coconut milk agar medium (Pallavi et al., 1997) and selected for detailed study. The culture of toxigenic *A. flavus* strain was maintained on potato dextrose agar slant in the lab.

Preparation of plant extract

Plant materials of five plant species belonging to five botanical families (Table 1) included in this study were collected, washed with tap water, disinfected by immersion in 2% sodium hypochlorite solution for 30 min, rinsed with sterile distilled water to eliminate residual hypochlorite and dried in shade. The shade-dried material of each plant species was grounded into a powdered material using a blender to pass 100 mm sieve and the mince was sealed in polyethylene bags until extraction. For preparation of methanolic extracts, 50 g of dry powder plant material from each plant species was soaked in methanol (10 ml of methanol/g of plant material) with stirring for 48 h then filtered through double layers of muslin, centrifuged at 9000 rpm for 10 min and finally filtered again through Whatman filter paper No. (41) to remove leaf debris and obtain a clear filtrate. The filtrates were evaporated and dried under reduced pressure and temperature below 40°C.

Antifungal screening test of plant extracts

Antifungal activity was evaluated on the toxigenic *A. flavus* strain using Czapek dox broth medium (sucrose, 30 g; sodium nitrate, 3 g; dipotassium phosphate, 0.5 g; magnesium sulfate, 0.5 g; potassium chloride, 0.5 g; ferrous sulfate, 0.01 g; distilled water, 1000 ml; pH, 6.5). The plant extract residues were re-dissolved in 5 ml of Czapek broth, sterilized in disposable Millipore filter (0.22 µm pores) and mixed with 45 ml of sterile Czapek broth in 150 ml Erlenmyer flasks to obtain final concentration of 10 mg ml^{-1} of each plant extract. The control set was kept parallel to the treatment sets without plant extracts. The flasks were inoculated with discs of 6 mm diameter of the toxigenic *A. flavus* isolate and incubated at 25 ± 2°C for 7 days.

After incubation, content of each flask was filtered (Whatman No. 1) and biomass of the filtered mycelium was determined after drying at 70°C for 4 days till their weights remains constant. The percentage of mycelial inhibition was calculated (Table 2) using the following formula:

Percentage of mycelial inhibition = [C – T / C] x 100

Where, C and T are the mycelial dry weight (mg) in control and treatment respectively.

Antifungal and aflatoxins inhibition assay

The effective plant extracts including *L. camara, O. europaea*, *P. granatum*, and *Z. officinalis* were used to determine minimal inhibitory concentration (MIC), minimal fungicidal concentration (MFC) and their anti-aflatoxigenic efficacy using yeast extract sucrose broth (YES) medium supplemented with 0.8% sodium chloride (Ezzat and Sarhan, 1991). Different concentrations of each plant extract (5, 10, 15, 20 and 25 mg/ml) were prepared separately by dissolving their requisite amount in 10 ml of YES broth, sterilized through Millipore filter and mixed with 40 ml of sterile YES broth in 150 ml Erlenmeyer flasks. Three replicates were performed for each concentration and the control set was kept parallel to the treatment without plant extract. The flasks were inoculated with discs of 6 mm diameter of the toxigenic *A. flavus* and incubated at 25 ± 2°C for 7 days. After incubation, content of each flask was filtered (Whatman, No. 1) and biomass of filtered mycelium was

Table 2. Antifungal screening of some plant extracts (10 mg/ml) against toxigenic *A. flavus* isolate.

Plant species	Mycelial dry weight (g)	Percentage of mycelial growth inhibition
Allium sativum L	0.655* ± 0.009	00.00
Lantanta camara	0.523* ± 0.004	19.54
Olea europaea	0.255* ± 0.008	60.77
Punica granatum	0.053* ± 0.002	91.85
Zingiber officinales	0.146* ± 0.002	77.54
Control	0.650* ± 0.005	00.00

Values in the same column followed by asterisk (*) are significantly different at (P = 0.05). Data are means (n = 3) ± standard error of three replicates.

dried at 70°C for 4 days till their weights remains constant. Mycelial dry weights of treatments and control was determined and MIC was calculated for each plant extract. For aflatoxins extractions, the filtrates of each flask were treated three times with 50 ml of chloroform in a separating funnel. The chloroform extract was separated and dehydrated with anhydrous sodium sulfate and evaporated till dryness on water bath at 50°C under vacuum. The residues were dissolved in 1 ml chloroform and 50 ul of chloroform extract spotted on TLC plates (20 x 20 cm^2) coated with 0.25 mm thick silica gel (Alugram, Germany). The plates were developed with benzene-methanol-acetic acid (95: 5: 5 v/v/v) solvent system described by (AOAC, 1995) and aflatoxin B_1 was detected by visual examination of TLC plates under UV lamp at 365 nm and comparison of the fluorescent band with that of the standard aflatoxin. The presence of aflatoxin B_1 was confirmed chemically by spraying trifluoroacetic acid (Bankole and Joda, 2004).

Quantification of aflatoxin B_1 was done by photodensitometry (Bio-metera- Germany) comparing the area and density of the spot samples with aflatoxin B_1 standard (Supelco - USA). Aflatoxin B_1 content was expressed in terms of μgl^{-1} and aflatoxin inhibition was calculated as follow; percentage of inhibition = [Y – X / Y] x 100, where X" is the concentration of aflatoxin in treated sample and Y" is the concentration of aflatoxin in control.

GC/GC-MS analysis of the effective plant extracts

Plant extracts of *O. europaea*, *P. granatum*, and *Z. officinalis* were analyzed according to the method described by Priyanka et al. (2009) through gas chromatography (Varian model, 450) equipped with a flame ionization detector, and quantitation was carried out by the area normalization method neglecting response factors. The analysis was carried out using a VF-5MS capillary column (30 m x 0.25 mm; 0.25 µm film thickness). The operating conditions were as follow: injection and detector temperature, 250 and 300°C respectively; split ratio, 1: 50; carrier gas, Helium with flow rate (1.0 ml/min). Oven temperature programme was 50 to 300°C at the rate of 7°C/min. Mass spectrometer conditions were: ionization potential, 70 eV; mass range from m/z, 40 to 400 amu; electron multiplier energy, 2000 V. The components of plant extracts were identified by comparison of their relative retention times and the mass spectra with those authentic reference compound shown in the literature (Adams, 2007) and by computer matching of their MS spectra with Wiley and Nist 8 mass spectral library.

Statistical analysis

All measurements were replicated three times for each treatment and the data were reported as mean ± SE (standard error). The data were also analyzed statically using one-way analysis of variance (ANOVA) and differences among the means were determined for significance at $P \leq 0.05$ using Duncan's multiple range test (by SPSS, 16.1 Chicago, USA).

RESULTS

The ethnobotanical data (botanical and local name) of the plants used in this study are shown in Table 1.

A total number of five plants from five different families were studied regard to their antifungal and antiaflatoxigenic activities against the toxigenic *A. flavus* isolate. Among the five methanolic plant extracts screened for their antifungal activity, *P. granatum* showed the highest antifungal activity at 10 mg/ml while the extract of *Z. officina*lis and *O. europaea* showed a moderate antifungal activity against the toxigenic *A. flavus* isolate. On the other hand, the methanolic extract of *L. camara* showed a weak antifungal activity and no effect was detected with *A. sativum* (Table 2). Hence, the methanolic extracts of *L. camara, O. europaea*, *P. granatum*, and *Z. officinalis* were selected for further investigation.

The MIC and MFC were employed by poisoned food technique to assess fungistatic and fungicidal properties of the most effective plant extract. As illustrated in Figure 1, the inhibitory plant extracts show various capabilities to suppress *A. flavus* grown on broth medium. Although, the inhibitory effect of the plant extracts increased in proportion to their concentrations and reached to a maximum in the final concentration of 20 mg/ml, the extract of *P. granatum* showed the highest growth inhibition (91.85%) at concentration 10 mg/ml followed by *Z. officinalis* (77.54%), *O. europaea* (60.77%) and *L. camara* (19.54%). This inhibition was reported to be significant for all plant extracts at the level of 0.05 (ANOVA). *P. granatum* was strongly active at MIC of 5 mg/ml and at MFC of 15 mg/ml while *Z. officinalis* showed a fungistatic activity against the toxigenic *A. flavus* with MIC of 10 mg/ml and MFC of 20 mg/ml. None of the tested plant extracts were able to suppress fungal growth at concentration of 5 mg/ml as shown in the Figure 1. As aflatoxin B_1 (AFB_1) is shown to be the most significant among all classes of aflatoxins in terms of

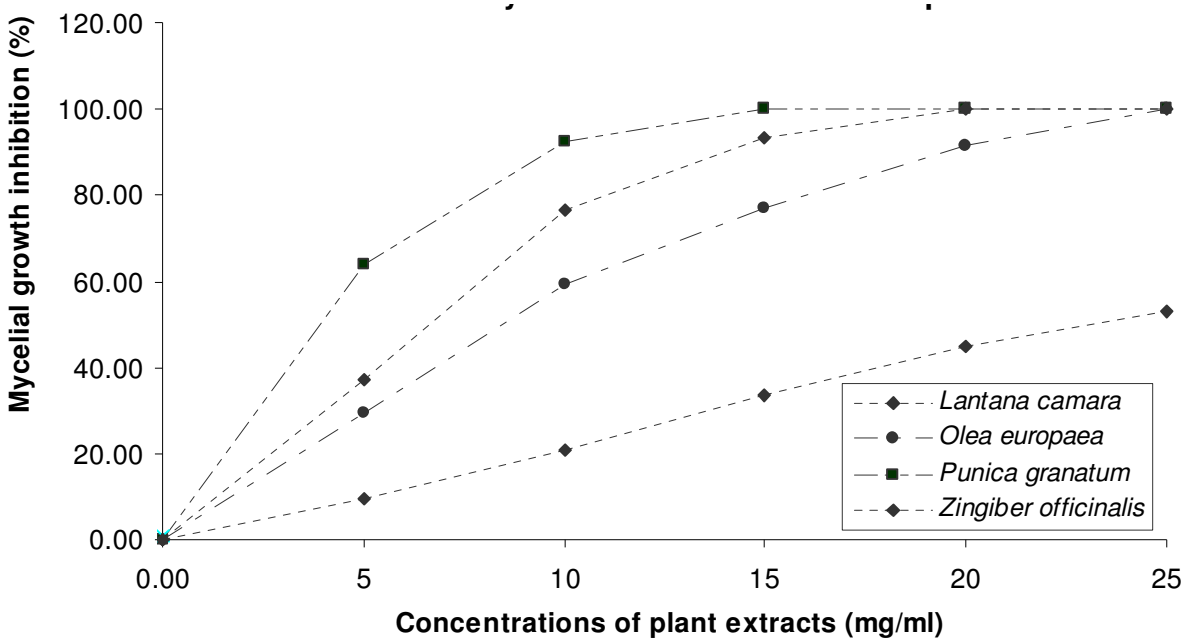

Figure 1. Percentage of mycelial growth inhibition of the toxigenic *Aspergillus flavus* isolate by different concentrations of plant extracts.

animal and human health risk, extracts of *P. granatum*, and *Z. officinalis* were found efficient to arrest the (AFB_1) production by *A. flavus* and completely inhibited at 5 and 10 mg/ml while *O. europea* absolutely arrested (AFB_1) production at 15 mg/ml (Table 3). A higher concentration of *L. camara* extract more than 25 mg/ml was required to inhibit growth and (AFB_1) produced by the toxigenic *A. flavus* isolate.

A direct correlation was found between fungal growth and AFB_1 production that is, a significant decrease in mycelial biomass resulted low AFB_1 production and vice versa. As a fungitoxicant, *P. granatum* extract was evaluated to be more efficacious fungitoxicant than the other plant extracts. The extract of *P. granatum* completely inhibited AFB_1 production by reduction mycelial growth with (64.02%) at concentration 5 mg/ml while the extract of *Z. officinalis* absolutely arrested AFB_1 production with biomass reduction of (76.86%) at a higher concentration 10 mg/ml. Although, *O. europaea* extract showed a moderate antifungal activity, the extract exhibited a significant antiaflatoxienic efficiency as it completely inhibited aflatoxin B_1 production at 15 mg/ml

The main constituents of the effective plant extracts, that is, *O. europaea, P. granatum* and *Z. officinalis* identified by GC-MS spectrometer analysis are summarized in Table 4 according to their retention indices (RI) and percentage composition. Anthracenedione (20.63%), terbutaline (13.96%) and propiolic acid (12.02%) were the principal components of *O. europaea* extract. Gingerol, cedrene, zingiberene, and α-curcumene were present in 46.85, 8.39, 7.41 and 7.32% respectively, in the extract of *Z. officinalis. P. granatum* extract was mainly composed by ellagic acid (37.01%), and ellagic acid derivatives like pedunculagin (6.40%), punicalagin (5.64%), granatin (5.1%) and polyphenol as lumicolchicine (4.68%).

DISCUSSION

Aflatoxins receiving the most attention as potential hazard to human and animal health and are known to be potent hepatocarcinogens. Therefore, the presence of toxigenic fungi and mycotoxins in foods and grains stored for long periods of time presents a potential hazard to human and animal health. In the present study, a toxigenic strain of *A. flavus* isolated from wheat seeds was chosen because of its toxigenic productivity and its strong affinity to colonize wheat seeds and various food commodities render them unfit for human consumption (Bandyopadhyay et al., 2007). Adverse effects of chemical pesticides on environment and human health are burning issues and there is a need to search for new fungicides with improved performance as well as eco-friendly in nature. Hence, some extracts of ethnomedicinally important higher plant species were tested for their antifungal activity. Such plant products would be biodegradable and safe to human health (Mohanlall and Odhav, 2006). In continuing of the studies on antifungal and aflatoxins inhibitors, a number of 5 plant species from five different families were evaluated for their antifungal effects against aflatoxin producing

Table 3. Antiaflatoxigenic efficacy of some plant extracts.

Plant extracts	Concent. (mg/ml)	Biomass (g)	Rf	Aflatoxin B1 content (µgl-1)	% Inhibition of aflatoxin B1
Lantana camara	0.00	0.724* ± 0.049	2.81	384.20	---
	5.00	0.655* ± 0.004	2.79	327.60	14.73
	10.0	0.574*± 0.004	2.79	294.80	23.27
	15.0	0.481* ± 0.004	2.81	228.40	40.55
	20.0	0.401* ± 0.003	2.78	182.80	52.42
	25.0	0.339* ± 0.002	2.82	106.20	72.36
Olea europea	0.00	0.745* ± 0.022	2.79	386.10	---
	5.00	0.527* ± 0.014	2.81	225.4	41.67
	10.0	0.304* ± 0.002	2.78	112.60	70.84
	15.0	0.173* ± 0.002	---	0.000	100.0
	20.0	0.062* ± 0.001	---	0.000	100.0
	25.0	0.000 ± 0.000	---	0.000	100.0
Punica granatum	0.00	0.706* ± 0.025	2.79	384.60	---
	5.00	0.254* ± 0.002	---	0.000	100.0
	10.0	0.055* ± 0.001	---	0.000	100.0
	15.0	0.000 ± 0.000	---	0.000	100.0
	20.0	0.000 ± 0.000	---	0.000	100.0
	25.0	0.000 ± 0.000	---	0.000	100.0
Zingiber officinale	0.00	0.687* ± 0.009	2.79	382.30	----
	5.00	0.432* ± 0.003	2.78	110.30	71.16
	10.0	0.159* ± 0.003	---	0.00	100.0
	15.0	0.047* ± 0.003	---	0.00	100.0
	20.0	0.000 ± 0.000	---	0.00	100.0
	25.0	0.000 ± 0.000	---	0.00	100.0

Values in the same column followed by asterisk (*) are significantly different at (P = 0.05). Data are means (n = 3) ± standard error of three replicates.

Table 4. Phytochemical composition of the most effective plant extracts and their relative contents (%).

Compound	Plant extracts					
	Olea europaea		*Punica granatum*		*Zingiber officinalis*	
	RI	%	RI	%	RI	%
2,3 Dihydroxypropanal	5.458	3.73	----	----	----	----
1,3 Dihydroxypropanone	7.511	2.29	----	----	----	----
Octamethylcyclotetrasiloxane	10.182	3.47	----	----	----	----
Decamethylcyclopentasiloxane	14.035	5.38	----	----	----	----
Methyl coumalate	17.529	2.63	----	----	----	----
9,10-Anthracenedione	17.612	20.63	----	----	----	----
4-Vinylguaiacol	18.033	2.59	----	----	----	----
4-Carbomethoxybenzaldehyde	19.161	13.56	----	----	----	----
Tyrosol	20.164	3.20	----	----	----	----
Terbutaline	23.517	13.96	----	----	----	----
α-Lumicolchicine	23.521	3.15	----	----	----	----
Phenylthioethanol	24.089	7.59	----	----	----	----
Propiolic acid	25.419	12.02	----	----	----	----
Octasiloxane	28.288	5.82	----	----	----	----

Table 4. Contd.

Threitol	----	----	7.652	2.03	----	----
Thymine	----	----	12.793	1.16	----	----
Gallic acid	----	----	14.314	3.68	----	----
Deoxyspergualin	----	----	14.479	4.18	----	----
Ellagic acid	----	----	16.309	37.01	----	----
Glucogallin	----	----	16.733	4.81	----	----
Guanethidine	----	----	17.324	1.10	----	----
Gallagic acid	----	----	19.320	5.54	----	----
Guanosine	----	----	20.461	4.93	----	----
Corilagin	----	----	23.527	4.47	----	----
Punicallin	----	----	28.005	5.93	----	----
Dibutyl phthalate	----	----	28.753	1.30	----	----
Pedunculagin	----	----	29.954	6.40	----	----
Granatin	----	----	31.703	5.10	----	----
Noradrenaline,TMS	----	----	33.216	2.33	----	----
Lumicolchicine	----	----	34.828	4.68	----	----
Punicalagin	----	----	36.262	5.64	----	----
α-Curcumene	----	----	----	----	21.230	7.32
Zingiberene	----	----	----	----	21.475	7.41
α-Farnesene	----	----	----	----	21.587	1.07
Cedrene	----	----	----	----	22.004	8.39
±Nerolidol	----	----	----	----	22.580	2.71
β-Guaiene	----	----	----	----	23.943	1.86
β-Eudesmol	----	----	----	----	24.341	4.37
β-Cedren-9- α-ol	----	----	----	----	24.888	6.71
α-Bisabolene epoxide	----	----	----	----	26.626	7.64
Longipinocarvol	----	----	----	----	27.454	5.34
Gingerol	----	----	----	----	33.250	46.85

A. flavus. The methanolic extract from *P. granatum* was effectively suppressed fungal growth followed by *Z. officinalis* and *O. europaea*. These results are in accordance with that of Pina-Vaz et al. (2004), Nguefack et al. (2004) and Kumar et al. (2008) who reported that *Z. officinalis* extract inhibited mycelial growth of *A. flavus* with (93.7%) at 1.5 µl/ml. On the contrary, no inhibitory effect was detected with *A. sativum* extract even at higher concentration more than 25 mg/ml. These findings are in agreement with those of Misra and Dixit (1979), Benkeblia (2004) and Bluma et al. (2008). A variation in fungitoxicity of the concerned plant extracts against the toxigenic *A. flavus* may be due to considerable variation in their constituents (Cavaleiro et al., 2006). The study of MIC of the fungitoxicants is necessary to find out their minimum amount to check the pest population and it would be helpful in saving unnecessary wastage of the pesticides. The MIC of *P. granatum* extract was comparatively lower than some earlier reported extracts that is, *Z. officinalis* and *O. europaea* (Nguefack et al., 2004; Kumar et al., 2008). As aflatoxin B_1 (AFB_1) is known to be the most significant in terms of animal and human health risk, *P. granatum* extract showed marked efficacy in arresting aflatoxin B_1 production by the toxigenic *A. flavus* isolate. The mycelial growth and AFB_1 production were recorded to decrease on increasing the concentrations of the plant extracts. It is evident from Table 3 that the extract of *P. granatum* was found to be more potential as antifungal and antitoxicant than *Z. officinalis* extract. It was found that at 5 mg/ml of *P. granatum* extract, mycelial growth was inhibited to (64.02%) and AFB_1 production was completely checked while AFB_1 production was completely suppressed and mycelial growth was inhibited to (76.86%) with *Z. officinalis* extract at concentration of 10 mg/ml.

So the results obtained revealed that *P. granatum* extract is more efficient as aflatoxin-inhibitor and its antiaflatoxigenic activity may be due to its effective anti-oxidative properties which could suppress aflatoxins biosynthesis. Generally, in order to reduce the aflatoxins production, mycelial growth must be below the threshold limit so that aflatoxins could not be produced. Compositional analysis of *P. granatum, Z. officinalis* and *O. europaea* extracts showed that polyphenolic compounds as ellagic acid, ellagitannins (punicalagin, granatin, castalagin) and gallic acid present in

P. granatum extract and other phenolic compounds such as gingerol, cedrene, zingiberene, and α-curcumene present in *Z. officinalis* extract as well as other phenolic compounds present in *O. europaea* play the vital role in growth and aflatoxin inhibition. These phenolic compounds inhibited one or more early rather than late steps in the AFB_1 biosynthesis pathway. Some researcher attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plant extracts and their components. This enables them to partition in the lipids of the fungal cell wall membrane and mitochondria, disturbing their structure and rendering them more permeable. Leaking of ions and other cell contents can then occur (Cox et al., 2000; Burt, 2004). Other researchers have suggested that antimicrobial components of the plant extracts cross the cell membrane interacting with the enzymes and proteins of the membrane, so producing a flux of protons towards the cell exterior which induces change in the cell and ultimately their death "that is, alter metabolic pathway" (Omidbeygi et al., 2007). The results of the present study revealed that some plant extracts have been emerged as safe alternatives to replace chemical fungicides and preservatives and can be used as eco-friendly fungicides. On the basis of present findings, extracts of *P. granatum* and *Z. officinalis* can be recommended as potentially effective and environmentally safer alternative fungicides to protect the spoilage of wheat grains from the toxigenic *A. flavus* and they should find a practical application as eco-friendly fungicides.

ACKNOWLEDGEMENTS

The authors extend their appreciation to Deanship of Scientific Research at King Saud University for funding the work through the research group project No: RGP-VPP-010.

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