

## USE OF POMEGRANATE PEEL AS AN ANTIFUNGAL AND ADSORBENT MATERIAL FOR THE REMOVAL OF TOTAL AFLATOXINS (IN VITRO)

Abo Hager, Amel A.; Gihan M. El Moghazy and M. A. Atwa  
Regional Center for Food and Feed. Agriculture Res. Cent. Giza.

### ABSTRACT

PP was evaluated for its ability to control the *Aspergillus flavus* growth and to adsorb its toxic metabolite (Total aflatoxin-TAF) from aqueous solutions as well. The study was performed to evaluate the influence of different inclusion rates of dried Pomegranate Peel (PP) (0.0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 and 3.0%) in the media used to count the total number of colony forming units (cfu) of *Aspergillus flavus*. Obtained data revealed that, inclusion of the examined substrate at 0.4% showed significant reduction of the total colony forming units compared to the control treatment (0.0%). Evaluation of various parameters i.e. adsorbent level (3, 6, 9, 12, and 15%), contact time (shaking time) 1, 2 and 4 hours, and TAF concentrations (382.11, 763.72, 1145.58 and 1527.44ppm) was also performed. Chemisorption indices were determined by HPLC analysis of extracts of the supernatants (extracted with chloroform). Adsorption abilities ranged from 27% to 99%. The maximum removal of TAF being 98.8%, took place at 15% PP present in solution (w/v) and 4hrs shaking time. Also, PP showed high adsorption abilities at increasing TAF concentration. PP efficiency in feed against aflatoxicosis should be verified further by in vivo tests.

It is recommended in the view of the results obtained in this study that, PP can be used in animal feeding as toxin binder as it has the ability to adsorb mycotoxins. Further biological and toxicological trials are required and more research work with animals at low level of toxins.

**Keywords:** *Aspergillus flavus* – Antifungal, Total Aflatoxin - Pomegranate Peel – Decontamination- Invitro- and Adsorbent

### INTRODUCTION

Corn is considered as one of the most important feed ingredient used in animal feeding, so its safety is a critical parameter for the safety of feed offered to animals. Moulds are the major threatening agent that may be present in corn causing physical (respiratory irritation) and (chemical (mycotoxin production) hazards. The main enhancing factors for fungal growth and mycotoxin production are both delayed or insufficient dryness process of the high moisture content harvested crop. (El Moghazy *et al.*, 2003). So preservatives are used in treating such grains for controlling both growth and mycotoxins production. Selection of the proper preservative is dependent upon several factors a) antimicrobial spectrum, b) antimicrobial activity, c) chemical and physical properties, d) relative toxicity, e) resistance development, f) organoleptic properties, g) economical considerations and i) a suitable procedure for analysis. (El Moghazy *et al.*, 2003).

With the increasing knowledge and awareness of aflatoxin as a potent source of health to both man and farm animals, a great deal of effort

has been made to completely eliminate the toxin or reduce its content in foods and feedstuffs to significant levels (*Rustom 1997*).

Control methods should be directed at either reducing the concentration of aflatoxin to safe levels or to produce nontoxic degradation products without reducing the nutritional value of the treated commodities (*Doyle et al., 1982*). Methods of control can therefore be classified into two main categories: (1) prevention of mold contamination and growth, (2) detoxification of toxic products.

Detoxification processes involve removing, degrading, destroying, or inactivating aflatoxins in commodities by physical, chemical, or biological removal methods, for a successful detoxification system, the process must be economically feasible, if it is to be applied practically (*Ellis et al., 1991*).

The FAO requirement for acceptable decontamination process: (1) destroy, inactivate or remove aflatoxins; (2) not to produce nor leave toxic and/ or carcinogenic, mutagenic residues in the final products or in food products obtained from animals fed on decontaminated feeds;(3) not to alter significantly the important technologic properties; (4) destroy fungal spores and mycelium that could proliferate and produce new toxins under favorable conditions (*Piva et al., 1995*).

Many adsorbents as activated charcoal, bentonite, zeolite, aluminum silicates, and yeast cell wall were tested for binding of several TAF both in vitro and in vivo. The degree of adsorption in vitro depends on the physical structure of the adsorbent, i.e. the total charge and charge distribution, the size of the pores and the accessible surface area. On the other hand, the properties of the adsorbent molecules, the mycotoxins, such as polarity, size, shape and-in case of ionized compounds- their charge distribution (*Alexander et al., 2001*).

PP is a numerous waste product in the juicing process. The pomegranate (*Punica granatum*) is a fruit-bearing deciduous shrub or small tree growing to 5-8 m tall. The pomegranate is native to the region from Afghanistan, Pakistan, Iran, Mediterranean region, India and USA (*Talcott et al., 2006*)

The name "Pomegranate" derives from Latin pomum "apple" and granatus "seeded" widespread root for "Pomegranate" comes from the Ancient Egyptian rmm, from which derive the Hebrew rimmon, and Arabic Rumman. This root was given by Arabs to other languages including Portuguese (*Pavey and Roy, 2003*)

The Qur'an mentions pomegranate three times, twice as an example of the good things of god creates, once as a fruit in the garden of Paradise.

The aim of this work is to evaluate the PP as antifungal as well as aflatoxin binder agent.

## MATERIALS AND METHODS

### Materials:

#### a) Pomegranate preparation:

Fresh PP was obtained from Juice processing factories in Cairo. The fresh PP distributed on trays and dried in open air, then transferred to an air circulation oven at 60°C for 16 hours. The dried PP was ground using a laboratory mill.

#### b) Aflatoxin production

Aflatoxins were produced by inoculating liquid yeast extract with one ml of fungal suspension contained  $10^6$  colony forming units (cfu)/ml of *Aspergillus flavus* spores (NRRL 3145) followed by incubation at 30°C for 10 days (Davis *et al.*, 1966). Mycelium mat was broken using glass rod and collected by filtration through filter paper. The filtrate, containing TAF (will be called in the present work, mother solution) was stored at 4°C for later use. Such technique yielded great amounts of AFs, especially B<sub>1</sub> and G<sub>1</sub>.

### Methods:

#### a) Studying the antifungal effect of PP:

A suspension of  $10^6$  cfu/ml of *Aspergillus flavus* (NRRL 3145) Yeast extract broth was prepared according to (Davis *et al.*, 1966). Ten folded serial dilution of this suspension was done using sterile buffer solution which then inoculated in 14 sets of sterile petri dishes. Each set was poured by about 15 ml of sterile Rose Bengal Chloramphenicol agar contained 14 different concentration of dried powdered PP (0%, 0.05%, 0.1%, 0.2%, 0.4%, 0.6%, 0.8%, 1%, 1.2%, 1.4%, 1.6%, 1.8%, 2% and 3%). After solidification, the poured plates were incubated at 25°C for 7 days after which all colony forming units were counted and recorded (NMKL 2005).

#### b) Studying the toxin binding effect of PP:

Five AF solutions were prepared by adding 0.0, 0.5, 1.0, 1.5 and 2.0 ml of the mother solution to 50ml of sterile distilled water which was then completed to 100 ml to obtain concentrations of 0.00, 3832.11, 763.72, 1145.58ppm of AF. Six levels of the adsorbent -the prepared PP- (0.0, 3.0, 6.0, 9.0, 12.0 and 15.0g) were added to each AF diluted solutions, resulted in 30 treatments. The tubes were mixed using a shaker for 1, 2 and 4 hrs, then the solutions were filtrated through filter paper. A volume of the filtrate was transferred to a separator containing chloroform. The contents of the separator were shaken 30-60 sec., the bottom layer (chloroform) was separated and concentrated to dryness under a slow stream of nitrogen and then redissolved in acetone/water (15:85v/v) Analysis of aflatoxins: Aflatoxins were determined using HPLC technique (Agilent 1100 Series U.S.A) with column C18, (Lichrospher 100 RP-18, 5  $\mu$ m $\times$ 25cm ) according to the following technique: The mobile phase consisted of water: methanol: acetonitrile (54:29:17,v/v/v) at flow rate of 1ml/ min. The excitation and emission wavelengths for all aflatoxins were 362 and 460 nm (Flores detector), respectively (Roos *et al.*, 1997).

**c) Determination of Cation Exchange Capacity (CEC):**

CEC was determined by Two-step procedure involved (i) saturation of cation exchange sites with Na at pH 8.2, 60% ethanol solution with 0.4N NaOAc- 0.1 N NaCl; (ii) extraction with solution so that the soluble Na from the excess saturation solution, carried over from the saturation step to the extraction step, may be deducted from the total Na to obtain exchangeable Na, which is equivalent Cation Exchange Capacity (Black, 1982).

**Table (1): Cation Exchange Capacity of Dried PP**

Item	meq/100g	Reference strong level
CEC	80	80-120

Meq/100g = milliequivalent/100 g sample

**Table (2): Proximate analysis of dried PP.**

Item (%)	Content
Protein	4.35
Ash	4.35
Fiber	15.87
Fat	1.62
Moisture	10.2
Silica (Si <sup>+</sup> )	0.080

**Figure (1): Proposed scheme for the surface charges of silica, mould spores and aflatoxin's molecules**



**Aspergillus flavus spore and AF charge Particles with positive charge**

**Silica particles with negative**

**d) Chemical composition were determined as described in (AOAC 2004).**

**e) Statistical analysis:**

Data were presented as mean and standard deviation values. Analysis of Variance (ANOVA) was used to compare between means of total aflatoxins concentrations, and levels of PP %. Dun can's post-hoc test was used to determine significant differences between the means when ANOVA test result is significant. Paired t-test was used to compare between shaking times. The significant level was set at P < 0.05. Statistical analysis was performed with SPSS 15.0 (Statistical Package for Scientific Studies) for Windows ( Geofferey and streines, 2003)

## RESULTS AND DISCUSSION

Using natural additives to control fungal growth with special concern to toxigenic fungi is an emerging approach in animal feeding instead of using chemical compounds which may have adverse effect on general health. The importance of this application is to prevent the health hazard of moulds as either mycotoxins' producers or respiratory irritant through its spores (El Moghazy *et al.*, 2003). Beside its effect as antimould, this approach is considered as a suitable physical method used for the removal of mycotoxins either in the feedstuff or from the intestinal tract as a nutritionally inert sorbent in the diet that can sequester mycotoxins and reduce their absorption. The utilization of mycotoxins – binding adsorbents is the most applied way to protect animals against the harmful effects of contaminated feed (Ramos *et al.*, 1996).

Data obtained in Table 3 illustrated that, increasing the inclusion rate of dried PP into the used culture media gradually decreased the total number of *Aspergillus flavus* colony forming units (consequently log). This reduction is numerically marked at 0.1% and 0.2% inclusion rate then became significant (one log reduction or more) at 0.4%. Numerical reduction only was noticed by increasing the inclusion rate of dried PP. Recently, natural products have proved to be an alternative to synthetic chemical substances. Laurylene *et al.*, 2006 stated that, the inhibitory capacity of dried PP is due to its content of tannin (9.33%) and polyphenolics which have toxic and astringent effect resulting of inhibition of the fungal growth due to absorption of the intercellular liquids. Also Krishnamurthy and Shashikala 2006 reported the inhibitory effect of dried PP on spore multiplication and also on its ability to produce AF. This may be attributed also to the negative charge present on the surface of dried PP as the effect of its silica content which combined with the positively charged fungal spores preventing it from multiplication and mycotoxin production (Iler 1979).

**Table (3): Effect of different concentrations of dried PP on total count of *Aspergillus flavus* spores after incubation period.**

Conc. %	0.0	0.05	0.1	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	3.0
Cfu/g	15	15	12	100	80	93	33	31	27	34	49	25	25	20
	x10 <sup>5</sup>	x10 <sup>5</sup>	x10 <sup>5</sup>	x10 <sup>4</sup>	x10 <sup>4</sup>	x10 <sup>4</sup>	x10 <sup>4</sup>	x10 <sup>4</sup>	x10 <sup>4</sup>	x10 <sup>4</sup>	x10 <sup>4</sup>	x10 <sup>4</sup>	x10 <sup>4</sup>	x10 <sup>4</sup>
Log	6.18	6.18	6.08	6.00	5.90	5.97	5.52	5.49	5.43	5.53	5.69	5.40	5.40	5.30

Data of the comparison of different dried PP concentrations' effect and different time of exposure on the percentage of bound AF concentrations and amount of unbound AF are illustrated in (table 4&5).

At PP 3%, after 1 hour, there was no significant difference among AF concentrations. After 2 hours, there was no significant difference between AF 763.72 ppm and AF 1145.58 ppm which showed the significantly highest mean adsorption values. There was no significant difference between AF 382.11 ppm and AF 1527.44 ppm which showed the significantly lowest mean values. After 4 hours, there was no significant difference among AF 382.11 ppm, AF 763.72 ppm and AF 1145.58 ppm which showed the

significantly highest mean adsorption values. AF 1527.44 ppm showed the significantly lowest mean value.

At PP 6%, *after 1 shaking hour*, AF 382.11 ppm showed the significantly highest mean adsorption value. There was no significant difference among AF 763.72 ppm, AF 1145.58 ppm and AF 1527.44 ppm which showed the significantly lowest mean values. *After 2 shaking hours*, there was no significant difference between AF 763.72 ppm and AF 1145.58 ppm which showed the significantly highest mean adsorption values. There was no significant difference between AF 382.11 ppm and AF 1527.44 ppm which showed the significantly lowest mean values. *After 4 shaking hours*, there was no significant difference among AF 382.11 ppm, AF 763.72 ppm and AF 1145.58 ppm which showed the significantly highest mean adsorption values. AF 1527.44 ppm showed the significantly lowest mean value.

At PP 9%, *after 1 shaking hour*, AF 382.11 ppm showed the significantly highest mean adsorption value. There was no significant difference among AF 763.72 ppm, AF 1145.58 ppm and AF 1527.44 ppm which showed the significantly lowest mean values. *After 2 shaking hours*, there was no significant difference among AF 382.11 ppm, AF 763.72 ppm and AF 1145.58 ppm which showed the significantly highest mean adsorption values. AF 1527.44 ppm showed the significantly lowest mean value. *After 4 shaking hours*, there was no significant difference among AF 382.11 ppm, AF 763.72 ppm and AF 1145.58 ppm which showed the significantly highest mean adsorption values. AF 1527.44 ppm showed the significantly lowest mean value.

At PP 12%, *after 1 shaking hour*, AF 382.11 ppm showed the significantly highest mean adsorption value. There was no significant difference between AF 763.72 ppm and AF 1145.58 ppm which showed lower values. AF 1527.44 ppm showed the significantly lowest mean value. *After 2 shaking hours*, AF 1145.58 ppm showed the significantly highest mean adsorption value. There was no significant difference between AF 382.11 ppm and AF 763.72 ppm which showed lower values. AF 1527.44 ppm showed the significantly lowest mean value. *After 4 shaking hours*, there was no significant difference among AF 382.11 ppm, AF 763.72 ppm and AF 1145.58 ppm which showed the significantly highest mean adsorption values. AF 1527.44 ppm showed the significantly lowest mean value.

At PP 15%, *after 1 shaking hour*, AF 382.11 ppm showed the significantly highest mean adsorption value. There was no significant difference between AF 763.72 ppm and AF 1145.58 ppm which showed lower values. AF 1527.44 ppm showed the significantly lowest mean value. *After 2 shaking hours*, AF 1145.58 ppm showed the significantly highest mean adsorption value. There was no significant difference between AF 382.11 ppm and AF 763.72 ppm which showed lower values. AF 1527.44 ppm showed the significantly lowest mean value. *After 4 shaking hours*, there was no significant difference among AF 382.11 ppm, AF 763.72 ppm and AF 1145.58 ppm which showed the significantly highest mean adsorption values. AF 1527.44 ppm showed the significantly lowest mean value.

It is clear from the presented data that, there was a gradual increase in the percentage of the adsorbed amount of AF by the increase of the exposure time by shaking, i.e the highest percentage of adsorption was obtained after 4 shaking hours.

Increasing the inclusion rate of dried PP into the liquid substrate increased the percentage of bound AF in all AF concentrations, in conclusion it was noticed that, there was a reduction of 98.8% of AF concentration obtained by shaking the distilled water contained 763.72 ppm of AF and 15% dried PP for 4 hours.

Gradual increase in the AF adsorption efficiency of dried PP was noticed in all used AF concentrations while this efficiency was decreased in the highest AF concentration (1527.44 ppm) at all exposure times and different levels of dried PP.

All these findings were attributed to the adsorption capacity of dried PP due to its negative charge which is due to the Silica content which is strong negatively charged element (Fig. 1) causing attraction (adsorption) of the strong positively charged AF particles (Iler 1979). Another factor causing this adsorption characteristic of dried PP is its high fiber content which is clear in table (2). The fiber content offers number of hydroxyl binding sites (Amel Abo Haggar, 2006).

**Table (4): The mean and standard deviation values for the percentage of total bounded aflatoxins by using PP**

AF conc.	382.11 ppm			763.72 ppm			1145.58 ppm			1527.44 ppm		
Time PP %	1 hr	2 hrs	4 hrs	1 hr	2 hrs	4 hrs	1 hr	2 hrs	4 hrs	1 hr	2 hrs	4 hrs
3	K	BEI	AGI	I	AE	AGI	K	AEI	AG	I	BEK	BG
	33.8 ± 2.6	40.9 ± 3.5	84.2 ± 1.8	31.2 ± 3.7	80.6 ± 2.8	89.5 ± 1.7	27 ± 2.6	80.4 ± 1.7	90.6 ± 4.2	27 ± 2.8	36.5 ± 3	71.2 ± 4.5
6	AJ	BI	AGI	BI	AE	AGI	BK	AEI	AG	BI	BEJ	BG
	43.5 ± 3.3	47.4 ± 4.6	82.6 ± 6.7	33.1 ± 4.1	80.6 ± 3	89.3 ± 4.7	32.3 ± 2.6	81.2 ± 3.5	89.9 ± 2.8	30.9 ± 2.8	45.3 ± 2.9	72.5 ± 4.4
9	AI	AEH	AGI	BI	AE	AGI	BJ	AEI	AG	BH	BEJ	BG
	59.3 ± 4.4	80.9 ± 3.8	84.2 ± 4.1	37.6 ± 2.8	82.1 ± 3.1	89.2 ± 2.6	41.2 ± 2	85.9 ± 2.7	89.6 ± 3.1	38.8 ± 2.2	46 ± 2.8	73.4 ± 3.6
12	AH	BH	AGH	BH	BE	AGI	BI	AEH	AG	CH	CEI	BG
	78.7 ± 4.1	79.8 ± 3.2	81.8 ± 4.7	60.4 ± 3.6	80.2 ± 3.2	89.9 ± 3.7	56 ± 4.1	91.1 ± 4.2	84.2 ± 3.9	41.3 ± 3.2	41.3 ± 4.1	74.6 ± 2.1
15	AH	BH	AGH	BH	BE	AGH	BH	AEH	AG	CH	CEH	BG
	81.5 ± 3.1	81.7 ± 3.2	81.7 ± 3.7	63.6 ± 4.1	86.3 ± 3.6	98.8 ± 4	63.3 ± 2.8	92.5 ± 3.1	89.5 ± 3.7	40.6 ± 3.5	70.5 ± 3.1	79.3 ± 2.9

**A,B,C and D (Comparison between AF concentrations): Means in the same row with different letters are significantly different at P ≤ 0.05**

**E,F and G (Effect of time): E: Significance from 1 hr – 2 hrs, and G: Significance from 1 hr – 4 hrs)**

**H,I, J, K and L (Comparison between levels of Pomegranate %): Means in the same column with different letters are significantly different at P ≤ 0.05**

**Table (5): The mean and standard deviation values of total aflatoxins concentration in ppm) that were not bounded by using PP**

AF conc. PP %	382.11 ppm			763.72 ppm			1145.58 ppm			1527.44 ppm		
	1 hr	2 hrs	4 hrs	1 hr	2 hrs	4 hrs	1 hr	2 hrs	4 hrs	1 hr	2 hrs	4 hrs
3	AH	BEH	DGH	BH	CEH	CGI	CH	BEH	BGJ	DH	AEH	AGH
	252.9	225.8	60.5	582.1	148.3	835.5	835.5	225	107.2	1115.3	970.2	440.2
	± 5.8	± 6.1	± 7.1	± 8.9	± 9.2	± 8.9	± 7.3	± 7.9	± 8.6	± 7.7	± 9.1	± 8.3
6	AI	BEI	DGH	BI	CEJ	CGI	CI	BEI	BGI	DI	AEI	AGI
	216	201.2	66.5	511	110.1	81.42	775.5	215.9	115.2	1055	835.5	420.4
	± 4.7	± 6.8	± 7.6	± 8.7	± 5.7	± 7.3	± 8.9	± 4.7	± 10	± 9.2	± 7.9	± 9.1
9	AJ	DEJ	DGH	BJ	CEI	CGI	CJ	BEJ	BGI	DJ	AEJ	AGJ
	155.5	73	60.5	476.7	136.8	82.38	674.2	162	119.1	934.9	824.4	405.6
	± 9.4	± 6.3	± 6.6	± 8.4	± 8.3	± 6.9	± 5.9	± 8.2	± 6.2	± 7.4	± 8.3	± 8.3
12	AK	DJ	DGI	BK	BEH	CGI	CK	CEK	BGH	DK	AEK	AGK
	81.4	77.4	71.5	302.4	151.2	77.28	504.7	99.5	181.5	897.2	722.8	388.5
	± 7.6	± 3.3	± 3.6	± 7.4	± 8	± 6.2	± 6	± 8.2	± 7.2	± 8.3	± 7	± 9.1
15	AL	DJ	DGI	BL	BEJ	CGH	CL	CEL	BGI	DL	AEL	AGL
	70.5	69.9	70.5	278.3	104.5	90.5	420.3	86.2	120.5	907	450.6	315.5
	± 4.6	± 8.1	± 6.1	± 5	± 6.9	± 7.3	± 3.6	± 7.4	± 8.7	± 4.5	± 8.1	± 10

A,B,C and D (Comparison between AF concentrations): Means in the same row with different letters are significantly different at  $P \leq 0.05$

E,F and G (Effect of time): E: Significance from 1 hr – 2 hrs and G: Significance from 1 hr – 4 hrs)

H,I, J, K and L (Comparison between levels of Pomegranate %): Means in the same column with different letters are significantly different at  $P \leq 0.05$

## REFERENCES

- Alexander, H.; F. Stefan; K. Othmar, and D. Hans (2001). Mycotoxin detoxification of animal feed by different adsorbents. *Toxicology Letters* 22:179-188.
- Amel A. Abo Hagggar, 2006. Use of Bagasse as an adsorbent material for the removal of total Aflatoxins ( in-vitro). *J. Agic. Sci. Mansoura, Univ.* 31 (7): 4205-4212
- AOAC (2004). Association of Official Analytical Chemists, Official Method of Analysis, 26<sup>th</sup> edition, Benjamin Franklin Station.
- Black, C.A. (1982). *Methods of Soil analysis. Part I.* Amr. Soc. Of Agron..
- Davis, N.D.; U.L. Dione and D. W. El-Dridye (1966). Production of aflatoxins B1 and G1 by *Aspergillus flavus* in a semisynthetic medium. *Appl Microbiol*, 14:378-380.
- Doyle, M.P .. , R. S. Applebaum; R.E. Brackett, and E.H. Marth (1982). Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities, *J. Food Prot.*, 45: 964 - 969.
- El Moghazy, Gihan, M.; M.F. Emara; Mervat, H. Yousf and Akila S. Hamza 2003. Antifungal activity of some organic acids used as feed preservatives. *J. Agic. Sci. Mansoura, Univ.*, 28 (12) 8053-8061.
- Ellis, W.O.; J.P. Smith, and B.K. Simpson (1991). Aflatoxin in Food: Occurrence, Biosynthesis, Effects on Organisms, Detection, and Methods of control. *Critical Reviews in Food Science and Nutrition*, 30 (3): 403 -439.
- Geoffery R.N, D.L. Streinel. 2003. *Statistics*, Third edition, B.C Decker Inc, Hamilton – London.
- Iler R. K., *The Chemistry of Silica* (Wiley, New York, 1979).



- Krishnamurthy YL, Shashikala J. 2006, Inhibition of aflatoxin B 1 production of *Aspergillus flavus* isolated from soya bean seeds by certain natural plant products. Lett Appl Microbiol. 2006 Nov;43(5):469-74.
- Laurylene C. V.; Fábio C. S.; Maria C. C. S.; Maria do Socorro V. P.; Jane S. H.; and Maria H. P. P. (2006). Minimum Inhibitory Concentration of Adherence of *Punica granatum* Linn (pomegranate) Gel Against *S. mutans*, *S. mitis* and *C. albicans*, *Braz Dent J* 17(3): 223-227 | 223
- NMKL 2005, Nordic Committee on food analysis. Enumeration of mould and yeast in food and feed. Report no. 98, 4<sup>th</sup> edition.
- Pavey, Don and Roy Osborne. 2003. Effect of (Punica granatum L.) polyphenols after ingestion of a standardized extract in healthy human volunteers". J. Agric. Food Chem. 54 (23): 8956-61 On colours 1528
- Piva, G., FP. F.Galvano; RD. A. Pietri, AP, and RD. A. Piva, (1995). Detoxification methods of aflatoxins. A review. Nutrition Research, 15 (5): 767 -776.
- Ramos, A.J.; J. Fink -Gremmels, and E. Hernandez (1996). Prevention of toxic effects of mycotoxins by means of nonnutritive adsorbent compounds. J.Food Prot. 59: 631 -641.
- Roos, A. H.: H. J. Van der Kamp, and E. C. Marley, (1997). Comparison of immunoaffinity columns for the determination of aflatoxins in animal feed and maize. Mycotoxin Research, 13:1-10.
- Rustom, I. Y. S, (1997) Aflatoxin in food and feed: occurrence, legislation and inactivation by physical methods. Food Chemistry, 59 (1): 57-67.
- Talcoot SU, Stohlawetz P, Rios J, Hingorani L, Derendorf H (2006). "Absorption, meta olism, and antioxidant effects of pomegranate

## استخدام قشر الرمان كمادة لمقاومة الفطريات و إدمصاص سموم الأفلاتوكسين معمليا

أمل عبد العزيز أبو حجر، جيهان محمد المغازي و محمد عبد المطلع عطوة.  
المركز الإقليمي للأغذية والأعلاف مركز البحوث الزراعية - الجيزة.

تم تقييم استخدام قشر الرمان كمثبط لنمو فطر الأسبرجيلس فلافوس وأيضاً لقدرته على إدمصاص سموم الأفلاتوكسين معمليا في محلول مائي. وقد شملت الدراسة تقييم تأثير استخدام مستويات مختلفة من قشر الرمان المجفف (0، 0.05، 0.1، 0.2، 0.4، 0.6، 0.8، 1.0، 1.2، 1.4، 1.6، 1.8، 2.0، 3.0%) مضافة إلى البيئة المستخدمة لتنمية الفطر. وقد أظهرت النتائج إنخفاض معنوي في العدد الكلي لمستعمرات الفطر النامية على البيئة المتخصصة و التي تحتوي على تركيز 0.4% من قشر الرمان مقارنة بعدد المستعمرات النامية على البيئة الخالية من الإضافة. وأيضاً تم تقييم تأثير عدة عوامل على إدمصاص سموم الأفلاتوكسين مثل كمية المادة المدمصة (3، 6، 9، 12، 15%) وزن / حجم، فترة تعرض سموم الأفلاتوكسين للمادة التي تقوم بالإدمصاص (1، 2، 4 ساعات رج) تركيز سموم الأفلاتوكسين الكلية (11، 382، 763، 1145، 1527، 44) جزء في المليون). وتم تقدير دلائل القدرة الكيمو إدمصاصية بواسطة تحليل الجزء الناتج عن الترشيح (والمستخلص بواسطة الكلوروفورم) بجهاز الفصل الكروماتوجرافي عالي الكفاءة.

تراوحت القدرة الإدمصاصية من 27% - 99% ووجد أن أقصى نسبة إزالة 98.75% عند 15% إضافة إلى المحلول (وزن / حجم) المحتوي على سموم الأفلاتوكسين بتركيز 763، 72 جزء في المليون مع الرج لمدة 4 ساعات. أيضا أظهرت قشور الرمان قدرة إدمصاصية عالية مع ارتفاع تركيز سموم الأفلاتوكسينات.

وعلى ضوء النتائج المتحصل عليها من هذه الدراسة يتضح إمكانية استخدام قشر الرمان في علائق الحيوان للحد من النمو الفطري وكذلك لتقليل التأثيرات السلبية للسموم الفطرية نظرا لقدرته الأدمصاصية العالية. وتأكيدا لذلك فإن هذا الاتجاه في حاجة إلى المزيد من الدراسات على الحيوان وعلى مستويات منخفضة من السمية.