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Aflatoxin B₁ concentration in wheat grain samples collected from the Egyptian local markets

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Abstract: Wheat (*Triticum vulgare* L.) samples were collected from different villages in three Centers of Minoufiya Governorate, Egypt during the period (2013-2014) and used immediately for Aflatoxin B₁ (AFB₁), moisture and fat content determination. AFB₁, moisture and fat content in samples were varied from 0.98 to 3.19 µg.kg⁻¹, 13.57 to 16.11% and 1.39 to 1.89%, respectively. Samples with the higher AFB₁ concentration are the samples of higher moisture and fat content. Half of the tested wheat grains samples recorded AFB₁ concentration more than the maximum permissible limits for human consumption (2 µg/kg AFB₁). When all wheat samples were included in the statistical analysis, there was a positive significant ($p \leq 0.05$) relationship between moisture content ($r^2 = 0.694$), fat content ($r^2 = 0.527$) and AFB₁ concentration. These correlations confirm that moisture content is mainly participate for the AFB₁ concentration of the tested wheat grain samples while fat content are partially participated. In conclusion, consumption of some wheat sampled stored by traditional methods can pose a potential risk of development of various diseases in human and animals. Also, for the proper storage of wheat grain, environmental factors such as moisture content and temperature must be controlled.

Keywords: *Triticum vulgare* L., moisture, fat, correlation analysis, storage traditional methods

Introduction

Wheat is among the important cereal crops of Egypt and are consumed in various ways by almost the entire population of the country. Wheat is exclusively cultivated as a winter crop in 3,378,659 Fadden with a production of 9 millions tones in the year 2015 (http://www.masrawy.com/News/News_Egypt/details/2016/1/4/726778/). Harvested grains are stored by farmers for considerable periods in various types of storage structures, usually made of mud, open shads, in canvas or plastic sacks. Earthenware containers (Swmaa) of different shapes and sizes are also used frequently to store grains including wheat. Such as mentioned by Nasr (1998), Shapira, (2004) and Suleiman *et al.*, (2013) these traditional storage methods inevitably provides suitable conditions for the growth and metabolism of the insects, rodents and microorganisms responsible for quality loss in stored grains.

A number of microorganisms including fungi have been reported to be associated with stored wheat and their products causing losses of food intended for human and animal consumption (Abdullah *et al.*, 2000; Shapira, 2004; Algirdas *et al.*, 2006; Laca *et al.*, 2006; Balazs and Schepers, 2007; Binder *et al.*, 2007; Agnieszka and Krzysztof, 2013). Indeed, four major species of fungi have been discovered belonging to the species of *Aspergillus*, *Fusarium*, *Penicillium*, and *Claviceps* that produced some major mycotoxins such as aflatoxin, ochratoxin A, fumonisim, and zearalenone (Paterson and Lima 2010; Mohd-Redzwan *et al.*, 2013). Aflatoxin can cause both acute and chronic toxicity in animals (Bennett and Klich, 2003; Barrett, 2005 ; Wu and Tritscher, 2011; Bommakanti and Waliyar, 2012). Effects such as acute liver damage, liver cirrhosis, liver cancers, induction of tumors and teratogenic and other genetic effects are well documented (Wu and Khlangwiset, 2010; Wu and Tritscher, 2011; Thrasher, 2012; USAID, 2012). Aflatoxin B1 (AFB1), the most toxic aflatoxin, is the most potent naturally occurring chemical liver carcinogen known. For people who are chronically infected with hepatitis B virus (HBV), aflatoxin consumption raises the risk of hepatocellular carcinoma (HCC; liver cancer) (Groopman *et al.*, 2005). Acute aflatoxicosis, characterized by hemorrhage, acute liver damage, edema, and death, can result from

extremely high doses of aflatoxin (Jiang *et al.* 2005, 2008, Turner *et al.* 2007; Khlangwiseta and Wua, 2011).

Therefore, the present work is a limited survey for determination the AFB₁ concentration in wheat grain samples collected from the Egyptian local markets. In line with recommended by the previous studies, the occurrence of stored grain AFB₁ is very much influenced by geographical and climatic conditions as well as environmental factors (Oyekale *et al.*, 2012; Agnieszka and Krzysztof, 2013 and Suleiman *et al.*, 2013; Nikolett *et al.*, 2015), moisture and fat content will be determined in these wheat grain samples to investigate their relationship with the grain AFB₁ concentration detected.

Material and Methods

Materials

Wheat samples (1000 g) were collected from different villages in three Centers, Al-Bagour, Tala and Shebin El-Kom of Minoufiya Governorate, Egypt during the period (2013-2014). The collected samples were taken out randomly, transported to the laboratory and used immediately for analysis. Plastic polyethylene pages, one kilogram volume, used in samples collection were purchased from the local markets, Port Said City, Port Said, Egypt.

Chemicals and reagents: Aflatoxin B₁ from *Aspergillus flavus* and trifluoroacetic acid (TFA) were purchased from Sigma Chemical Co., St. Luis, MO. All other reagents and solvent were of analytical or HPLC grade were purchased from (Fisher, UK). De-ionized water (Milli-Q 18.2 MΩ) was used in the preparation of the mobile phases, reagent solutions and standards.

Methods

Determination of moisture and fat content

Wheat samples were analyzed for moisture and fat (Soxhelt mini-automatic apparatus Velp Company, Italy, petroleum ether solvent) were determined using the methods described in the A.O.A.C. (1995).

Determination of AFB₁ by HPLC

Sample extraction: Weigh 50g sample with 10g salt sodium chloride and place in blender jar (El-Araby, Toshiba, Benha, Egypt). Add to jar 200 ml methanol: water (80:20). Cover blender jar and blend at high speed for 1 minute. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

Extract dilution: Pour 10 ml filtered extract into a clean vessel. Dilute extract with 40 mL of purified water and mix well. Filter dilute extract through glass microfiber filter into a glass syringe barrel using markings on barrel to measure 4 ml.

Sample elution: Pass 4 ml filtered diluted extract (4 ml= 0.2g sample equivalent) completely through AflaTest ®-P affinity column (VICAM , Watertown, MA) at a rate of about 1-2 drops/second until air comes through column. Pass 5 ml of purified water through the column at a rate of about 2 drops/second. Elute affinity column by passing 1.0 ml HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1ml) in a glass vial. Evaporated to dryness under stream of nitrogen and was determination of HPLC.

AFB₁ Derivatization: The derivatives of samples and standard were done as follow: 100 µl of trifluoroacetic acid (TFA) was added to samples and mixed well for 30 s and the mixture stand for 15 min. 900 µl of water: acetonitrile (9:1 v/v) were added and mixed well by vortex for 30s .and the mixture was used for HPLC analysis.

HPLC analysis: Throughout this study a SP Thermo Separation Products Liquid Chromatography (Thermo Separation products, San Jose, CA) was used with a Consta Metvic 4100 pump, a Spectra Series AS100, Spectra System UV 1000 UV/Visible Spectrophotometer Detector, Spectra System FL 3000 and a PC 1000 system software. The columns used (PerkinElmer, Inc., Waltham, MA) were a C18 (3 µm, 100 x 4.6 mm I.d.) for AFB₁. An isocratic system mentioned by Troiano and Reuter (2007) was used for AFB₁ separation as follow: Mobile Phase: Isocratic: 60:10:30 Water/ACN/MeOH, with 119-mg potassium bromide and 350-µL 4M HNO₃ , Flow rate: 1.2 mL/min, Temperature: Ambient, Fluorescence Detector: Ex₃₆₂ nm and Em₄₃₅ nm and Injection Volume: 100 µL.

Statistical Analysis

All measurements were done in triplicate and recorded as mean±SD. Statistical analysis was performed with the Student *t*-test and MINITAB 12 computer program (Minitab Inc., State College, PA).

Results and Discussion

Data in table (1) and figure (1) showed the AFB₁ concentration, moisture and fat content in wheat grain samples collected from the Egyptian local markets during the period (2013-2014). From such data it could be noticed that the AFB₁ concentration, moisture and fat content were varied from 0.98 to 3.19 µg.kg⁻¹, 13.57 to 16.11% and 1.39 to 1.89%, respectively. A significant variation in AFB₁ was observed amongst the samples tested which clearly indicated seasonality factor affected. Seasonality factor could be included the differentiation of temperature, relative humidity, pest activity etc. Also, samples with the higher AFB₁ concentration are the samples of higher moisture and fat content. The European Union has enacted a very stringent aflatoxin tolerance threshold of 2 µg/kg aflatoxin B₁ and 4 µg/kg total aflatoxins for nuts and cereals for human consumption (Bankole and Adebajo, 2003). Therefore, half of the tested wheat grains samples recorded AFB₁ concentration more than the maximum permissible limits for human consumption. Consumption of such aflatoxin-contaminated samples can pose a risk of development of various diseases in human and animals.

Previous investigations showed that grains including wheat could be contaminated by aflatoxins above the limits that may be critical for health. For example, Vargas *et al.*, (2001) reported that 38.3% of maize samples were contaminated with aflatoxin B₁ with a mean of 9.4 µg/kg and a maximum of 129 µg/kg. High aflatoxin levels in maize, in some other African countries, notably Benin and Togo have been reported and one third of the household grain, contained aflatoxins in the range of five-fold the safe limit (Wagacha and Muthomi, 2008).

The largest and the most severe documented aflatoxin poisoning has been reported at a level as high as 8,000 µg/kg in Kenya in 2004, causing 125 deaths

Table 1. AFB₁ concentration, moisture and fat content of wheat grain samples collected from the Egyptian local markets during the period (2013-2014)

Wheat sample (n-3)	AFB ₁ concentration (µg.kg ⁻¹)	Moisture content (%)	Fat content (%)
Batch 1 (May, 2013)	0.98 ± 0.55 ^{*d}	13.90 ± 0.30 ^d	1.45 ± 0.14 ^c
Batch 2 (June, 2013)	2.32 ± 0.62 ^b	14.59 ± 1.02 ^c	1.57 ± 0.20 ^b
Batch 3 (July, 2013)	3.07 ± 1.03 ^a	15.68 ± 1.24 ^b	1.89 ± 0.13 ^a
Batch 4 (August, 2013)	2.54 ± 0.95 ^{ab}	15.41 ± 0.98 ^b	1.63 ± 0.22 ^b
Batch 5 (September, 2013)	2.21 ± 0.84 ^b	14.23 ± 1.11 ^c	1.60 ± 0.15 ^b
Batch 6 (October, 2013)	3.19 ± 1.17 ^a	16.11 ± 1.19 ^a	1.76 ± 0.21 ^a
Batch 7 (November, 2013)	1.46 ± 0.64 ^c	14.09 ± 0.56 ^c	1.52 ± 0.13 ^{bc}
Batch 8 (December, 2013)	2.53 ± 0.11 ^{ab}	14.27 ± 1.09 ^c	1.68 ± 0.16 ^{ab}
Batch 9 (January, 2014)	1.09 ± 0.39 ^d	13.89 ± 0.24 ^d	1.61 ± 0.13 ^b
Batch 10 (February, 2014)	1.98 ± 0.60 ^b	14.33 ± 0.38 ^c	1.54 ± 0.27 ^{bc}
Batch 11 (March, 2014)	1.21 ± 0.79 ^c	13.88 ± 0.33 ^d	1.56 ± 0.17 ^{bc}
Batch 12 (April, 2014)	1.03 ± 0.57 ^d	13.57 ± 0.19 ^d	1.39 ± 0.32 ^c

* Each value represents the mean of three replicates ±SD, values with the different letters in the same column are significant at level p≤0.01

out of 317 case-patients (Wagacha and Muthomi, 2008). Finally, fifty-one maize samples, intended for animal feed and human consumption, were collected from the four main maize production provinces in Iran and analyzed for aflatoxins. AFB₁ was detected in 58.3%, and 80% of the maize samples obtained from Kermanshah and Mazandaran provinces, respectively (Yazdanpanah, 2006 and Ghiasian *et al.*, 2011).

On the other side, data of the present study indicated that a significant variation in AFB₁ concentration detected in wheat grain samples. Such variations could be attributed to the effect of AFs production factors behind each sample. Previous studies indicated that AFs production is the consequence of a combination of species, substrate and environment. The factors affecting AFs production include temperature, pH, relative humidity of the atmosphere, water activity, moisture, light, aeration and level of atmospheric gases (Abramson *et*

al., 1998; Mehrdad *et al.*, 2011 and Felizardo and Câmara, 2013). AFs production in the substrate can happen in the field and in storage conditions between 20 and 40 °C with a 10- 20% of moisture and 70-90% of relative humidity in the air (Raila *et al.*, 2006). Delayed drying as well as high moisture content and crop storage can cause postharvest contamination. High levels of aflatoxin B₁ contamination in rain-affected maize and rice at a level of 15600 and 1130 µg/kg respectively, was reported (Vasanthi and Bhat, 1998 and Mehrdad *et al.*, 2011). The development of the fungus producing AFs is favored if the grains are damaged by insects or rodents. Same spores of the substrate bud and grow as mycelia generators of AFs because, when breathing, they produce water increasing the humidity of the grains (Frisvad, 1995 and Mehrdad *et al.*, 2011).

In the correlation analysis, important differences were found between moisture and fat content and AFB₁ detected in wheat grain samples collected from the Egyptian local markets (Figures 2). When all wheat samples were included in the statistical analysis, there was a positive significant ($p \leq 0.05$) relationship between moisture content ($r^2 = 0.694$), fat content ($r^2 = 0.527$) and AFB₁ concentration. These correlations confirm that moisture content is mainly participate for the AFB₁ concentration of the tested wheat grain samples while fat content are partially participated. Also, these data indicates that many other environmental factors beside moisture and fat content including relative humidity, temperature, growth of microorganisms and insects in the grains (Oyekale *et al.*, 2012; Agnieszka and Krzysztof, 2013 and Suleiman *et al.*, 2013; Nikolett *et al.*, 2015). Our data was confirmed by Chang and Markakis (1982), in the event of AF contamination, moisture content of 16% or higher are hazardous in

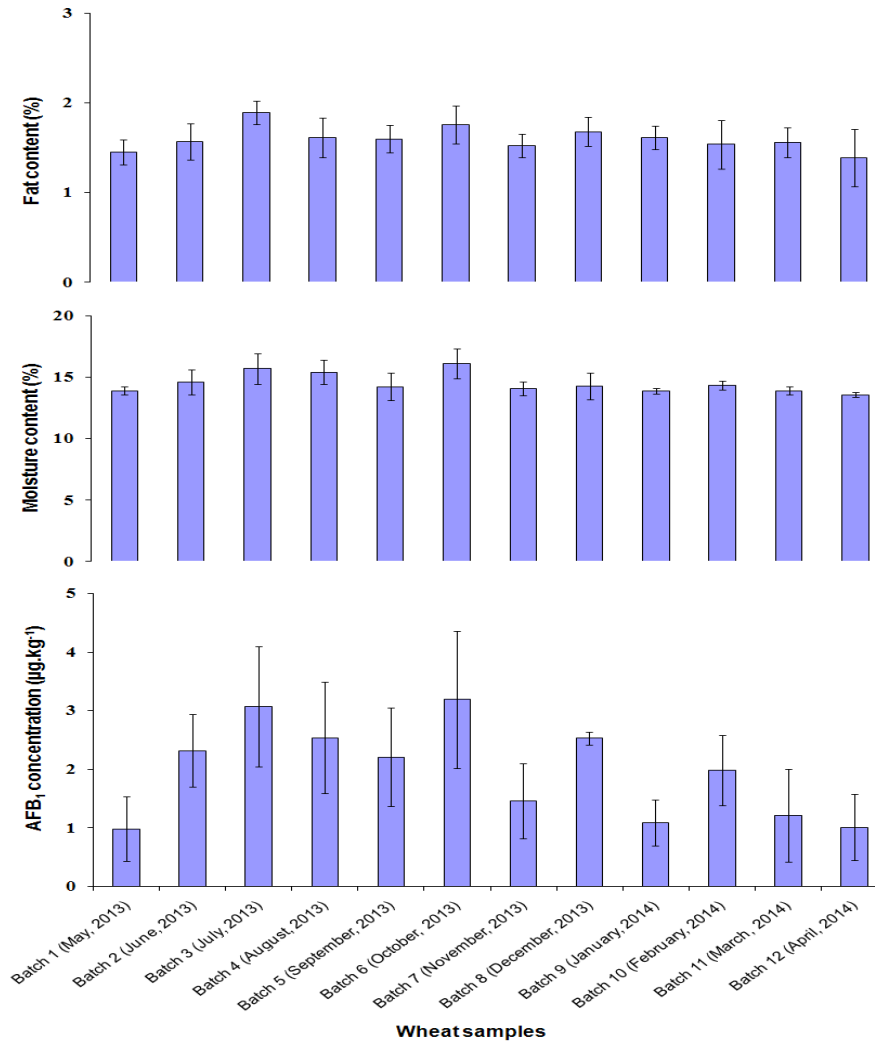


Figure 1. AFB₁ concentration, moisture and fat content of wheat grain samples collected from the Egyptian local markets during the period (2013-2014). Each value represents the mean of three replicates \pm SD.

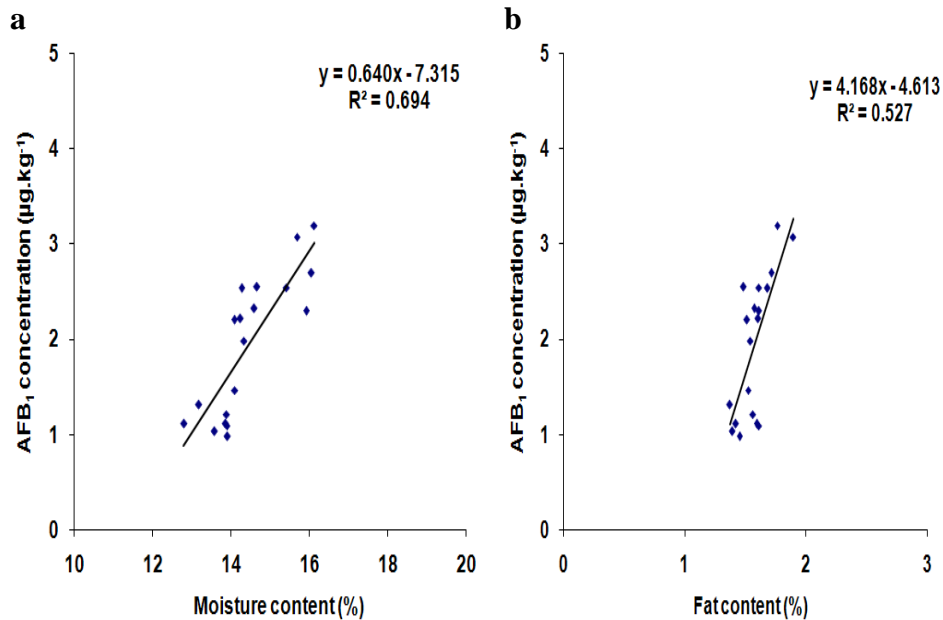


Figure 2. Correlation between moisture content, fat content and AFB₁ concentration detected in wheat grain samples collected from the Egyptian local markets during the period (2013-2014): (a) AFB₁ vs. moisture content, and (b) AFB₁ vs. fat content.

the storage of grains at temperatures near 25⁰C. Also, Agnieszka and Krzysztof (2013) stated that harvesting high moisture grain including wheat has become, however, common practice to protect the grain from wet weather conditions which can cause weathering and mould infection of grain in the field. High moisture grain is susceptible to deterioration by microorganisms including fungi produced AF and hence should be dried before unacceptable quality loss occurs. A 13 % moisture content is considered to be the maximum value for the storage of different grains including wheat, corn, barley and rice during short periods, to avoid spoilage of grain with fungi (Laca *et al.*, 2006). Regarding the relationship between the grain fat content and AFB₁ formation, dearth information is available. To interpret such relationship further studies in the future are required. In addition to the grain moisture and fat content, Oyekale *et al.*, (2012) confirmed that to

maintain high quality maize during storage, maize should be protected from weather (including relative humidity and temperature), growth of microorganisms and insects.

Conclusion

In conclusion, for the proper storage of wheat grain, environmental factors such as moisture content and temperature must be controlled. Such factors are the major influences of wheat deterioration, because they affect fungi growth and produce toxin such as aflatoxins. Relationship was observed between grain initial fat content and AFB₁ formation, but interpretation of such point will require further studies.

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تركيز الأفلاتوكسين ب₁ في عينات القمح المجمعة من الأسواق المحلية المصرية

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تم جمع عينات القمح من ثلاثة قرى مختلفة بحافظة المنوفية، جمهورية مصر العربية، خلال الفترة (٢٠١٣-٢٠١٤) وقد تم استخدامها على الفور لتقدير نسبة تركيز الأفلاتوكسين ب₁، والرطوبة ومحتوى الدهون. وقد تراوحت نسبة تركيز الأفلاتوكسين ب₁ من ٠,٩٨ إلى ٣,١٩ ميكرو جرام/كجم، وتراوحت درجات الرطوبة من ١٦,١١ إلى ١٣,٧٥%، وتراوح محتوى الدهون من ١,٣٩ إلى ١,٨٩ على التوالي. كما وجد ان العينات التي زاد فيها تركيز الأفلاتوكسين ب₁ كانت الأعلى في محتوى الرطوبة والدهون. وقد وجد أن نصف عينات القمح التي خضعت للأختبار قد سجلت أعلى من التركيز المسموح به للأفلاتوكسين ب₁ لإستهلاك الإنسان (٢ ميكرو جرام/كجم). وعند اجراء التحليل الإحصائي للعينات وجد ان هناك علاقة طردية / ايجابية بين محتوى الرطوبة ($r^2=0,694$)، نسبة الدهون ($r^2=0,527$)، وتركيز الأفلاتوكسين ب₁. وهذا الترابط يوضح مدى العلاقة بين تركيز الرطوبة الذي يؤثر بشكل رئيسي على معدل تركيز الأفلاتوكسين ب₁ في عينات القمح المختبرة في حين ان محتوى الدهون يؤثر تأثيرا جزئيا على معدل تركيز الأفلاتوكسين ب₁. وبذلك نكون قد توصلنا إلى أن استهلاك عينات القمح المخزنة بالطرق التقليدية تشكل خطورة كبيرة على صحة الإنسان والحيوان وبالتالي فإنه يجب أخذ المؤثرات البيئية كالرطوبة ومحتوى الدهون ودرجة الحرارة عند التخزين السليم لحبوب القمح في عين الإعتبار.

الكلمات المفتاحية: القمح – الرطوبة – محتوى الدهون – تحليل الارتباط – الطرق التقليدية للتخزين.