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ORIGINAL ARTICLES

Detection of Fungi and Mycotoxin Affected Wheat Quality

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ABSTRACT

Mycological survey was carried out on freshly harvested wheat grains from the main production regions i.e. Qalubya, Dekahlya, Gharbya and Menufya Governorates, Egypt yielded 460 fungal isolates. Results of two tested standard methods i.e. blotter test and agar plate presented that, agar plate (PDA medium) gave higher values and colony percent of fungi associated with wheat grains in all tested samples than blotter test which recorded 58.7 and 41.3 % respectively. Also, disinfection of the tested wheat grains lead to higher percentage of germinated seeds in both agar plate method and blotter test comparing with non-disinfected grains. On the other hand, the infection percent was higher with non-disinfected seeds compared with disinfected seeds. Eight fungal genera were isolated and identified as: *Alternaria 36.9 %, Aspergillus 12.4, Drechslera 1.3, Epicoccum 0.7, Fusarium 5.2, Mucor 0.2, Penicillium 18.3* and *Rhizopus* 25.0%. Analysis of mycotoxin resulted that, four fungal isolates were found to produce mycotoxins, i.e. two isolates of *A. flavus* (No.1&10) isolated from Qalubya and Gharbya samples, one isolate of *A. parasiticus* (No.54) from Menufya sample and one of *Fusarium moniliforme* (No.19) isolated from Dekahlya grain samples had the ability to produce different mycotoxins (i.e. aflatoxins B_1, B_2, G_1 and G_2 and fumonisin FB_1). Also; The obtained results revealed that contaminated wheat samples by *A. flavus*, *A. parasiticus, Fusarium moniliforme* and *Penicillium* sp. had higher crude protein, ash and fat but lower moisture, crude fiber and carbohydrates than uncontaminated. Contaminated samples were not formed gluten due to damaged gluten fractions comparison with control sample (uncontaminated). Also, contaminated samples were darker in color. Data of Hunter instrument indicated that contaminated samples had higher L. a. b values and the rate of color differences" E " compared to control. The highest color differences was recorded with *A. flavus*. On the other hand, the farinographic and extensographic curves show that the control sample had higher water absorption, mixing time, and dough weakening and lower dough stability, extensibility and dough energy, while the contaminated samples were not gave farinograms and extensograms due to breakdown of dough proteins and starch as a results of the action of protolytic and amylolytic enzymes which produced by fungi. Treated wheat grains by using some physical and chemical methods (i.e. hot air, ozone and sodium hypochlorite) were found to be reduced significantly total fungal colonies as well as increasing percentage of germinated seeds than un-treated with all tested wheat grain samples.

Key words: Mycoflora, Mycotoxins, Wheat Quality.

Introduction

Wheat (*Triticum aestivum* L. em Thell.), family *Poaceae* (*Gramineae*) is the most important staple food of about two billion people (36% of the world population). Worldwide, wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally**.** Among various factors that affect seed health, the most important are the seed borne fungi that not only lower seed germination, but also reduce seed vigor resulting in low yield. Healthy seed plays an important role not only for successful cultivation but also for increasing yield of crop (Wiese, 1984). Several seed-borne pathogens are known to be associated with wheat seed which are responsible for deteriorating seed quality during storage (Doohan *et al.*, 2003).

A total of five fungal species viz., *Alternaria tenuis, Aspergillus niger, Stemphylium herbarum, Fusarium moniliforme,* and *Curvularia lunata* were isolated from the seeds of 12 wheat varieties (Aslam, *et. al.*, 2005). A total number of 30 species of fungi viz., *A. candidus*, *A. flavus*, *A. fumigatus*, *A. parasiticus*, *A. niger, A. restrictus*, *A. sulphurus*, *A. sydowi*, *Alternaria alternata*, *A. brassicae*, *A. humicola*, *A. solani*, *Rhizopus oryzae*, *R.* spp., *R. stolonifer*, *Acremonium* spp., *Geotrichum candidum*, *Mucor heimalis*, *M. spp.*, *Cochliobolus lunatus*,

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Fusarium spp., *F. culmorum*, *Rhizoctonia*, *Curvularia lunata*, *Cladosporium herbarum*, *Penicillium frequentus*, *Botrytis* spp., *Nigrospora* spp., *Humicola*, *Helminthosporium* spp. were isolated from wheat grains (Maliha, *et. al*., 2010). Also, several fungi were isolated and recorded by many investigators i.e. (Attitalla, *et. al.* 2010 and Fatma Bensassl, *et al.,* 2011).

These pathogens may affect the plant resulting in a reduction of the grain quality; some species can even release natural toxins that cause human and animal intoxication. Mycotoxins prevention is very important as, once developed, they become stable at environment temperature and very resistant to thermal changes (Scussel, 2002; Santos, 2002; Garcia *et al.,* 2003 and Scudamore, 2005).

Compositional changes, rheological and technological properties of stored wheat grains due to infection with fungi were studied by several investigators. Dexter, *et al.*, (1996) reported that gluten originating from Fusarious wheat had lower content of glutenin fraction in comparison to gluten of healthy wheat .On the other hand, (Barabara, *et al .,* 2004 and Chandra, *et. al*., 2011) mentioned that the damage caused by fungi adversely affects the quality of wheat and reduces its nutritional composition. (Jackowiak, *et al.,* 2005), reported that *Fusarium* caused damaged of wheat kernels. Infected kernels revealed presence of fungal hyphae in the endosperm and some characteristic structural changes in many of its regions , such as partial or complete lack of the protein matrix, damage to large and small starch granules caused by fungal amylolytic enzyme, disappearance of small starch granules as the colonization progressed, complete disappearance of the starchy endosperm under severe infection. In a previous study (Marija, *et al.*, 2004), the researcher detected a higher contribution of major mycotoxigenic moulds in flour, making the product more susceptible to the accumulation of mycotoxins. Improved microbial quality and safety of food products and ingredients limit the risk of food borne illnesses and intoxications.

Unlike other commonly used disinfectants, ozone directly lyses the cell by attacking the cell membrane; there is no need for the biocide to be transported across the membrane and into the cell (Pryor and Rice 1999). Previous experiment have been demonstrated that the use of ozone for the purpose of microbial decontamination has widespread application. Ozone has the ability to oxidise organic material including the cell wall of an organism. Ozone can be used as a relatively brief pre-storage or storage treatment in air or water, or as a continuous or intermittent component of the atmosphere throughout storage or transportation (Palou, L. *et. al.,* 2001 & 2002). Ozone is widely used as an antimicrobial agent for bacteria, fungi, viruses and protozoa (Whangchai, *et. al.,* 2006).

Heat treatments have also been very effective in controlling fungi that are the main causes of postharvest decay development (Vicente *et al*., 2002). Heat treatment against pathogens may be applied to freshly harvested produce in several ways: by vapour heat, hot dry air, hot water dips (or a short, hot water rinsing and brushing (Fallik, 2004).

The aim of this work:

Survey of natural fungi contaminated wheat grain samples collected from four different localities. Identification of all fungal colonies appeared. Calculated both percentage of total fungal colonies and fungal frequencies which contaminated these samples. Detection of natural mycotoxins. Also, investigate the changes of chemical, physical and rheological properties of wheat grain which infected by some mold fungi. Decontamination of these fungi by some physical and chemical methods (a physical and chemical prevention means to reduce the mycological pollution of grain surface leads to enhanced the wheat quality).

Materials and Methods

1- *Survey:*

A survey was carried out during 2011 harvesting season in wheat growing districts area in Egypt. Four agro-ecological zones in Delta i.e. Qalubya, Dekahlya, Gharbya and Menufya Governorates were selected. Wheat grain samples (2kg per farm) were collected from each zone for mycological studies, mycotoxin analysis, physico-chemical changes and mycoflora decontamination. Samples were tacking according to (Mathur, *et. al*., 2003).

2- *Mycological study:*

a- Isolation and identification of fungi associated with wheat grains:

Each sample was thoroughly mixed and sub-sample taken randomly for mycological analysis by plating sterilized and un-sterilized wheat seeds by using tow standard testing methods namely blotter test and potato dextrose agar (PDA) medium (Mathur, *et. al*., 2003). The seeds of first group were surface sterilized as

described by Maliha *et al.*, 2010. Surface sterilized and un-sterilized kernels were plated on each plate containing low strength Potato Dextrose Agar (PDA) medium amended with antimicrobial agent (50 mg streptomycin) to suppress growth of bacteria (Muthomi, 2001). Others were plating them in sterilized Petri dishes with three layers of blotter papers moistened with sterilized water. All plates were incubated at 25±2ºC for 5-7 days, after which the number of kernels showing fungal infection were recorded. Fungi were identified to genus level following descriptions given by (Maren, & Johan, 1988 for genus *Aspergillus*, Nelson *et al.,* 1983 for *Fusarium*, and Barent & Hunter 1977 for the genera of imperfect fungi and Singh, *et al.,* 1991 for either Aspergilli, Fusaria and Penicillia) based on cultural characteristics and spore morphology. The relative isolation frequency (Fq) of each genus was calculated as recorded by Fatma Bensassi, *et al*., 2011.

b- Seed germination:

Seed germination were conducted by method described by Aslam, *et.al.*, 2005. All tests were replicated three times**.** The number of germinated grains and fungal colonies were counted daily.

$$\text{Germination (\%)} = \frac{\text{Number of germinated grains}}{\text{Total number of grains}} \times 100$$

Colonies growing from the kernels were sub-cultured on (PDA). All isolated fungi were recorded according to (Gonzalez *et al.,* 1999).

3- Mycotoxin production:

Sub samples of wheat grains were prepared in Food Toxicology and Contamination Dept. National Research Center (NRC) for detection of aflatoxins i.e. (AFB_1, B_2, G_1, G_2) and fumonosin (FB) according to the method described by (AOAC, 2005). Preparation of column chromatography according to (Ogido *et. al.,* 2004, and Wang, *et al.,* 2006). The HPLC system used in this work was a Jasco PU-1580 solvent delivery system and a MD-1510 UV/VIS detector, with a 10 µl flow cell. A reversed-phase Nucleosil C18 column (25 cm x 0.4 mm, µparticle size) was used for separation. A Reodyne 7725 injector with a 10 µL external loop was used for sample introduction. A Borwin chromatography workstation (system control version 1.5) was used to control the operation of HPLC, obtain the chromatogram, and perform data calculation. The working conditions were: Debit: 0.5 mil/min, Detector UV/VIS 218 nm, eluent: water/ acetonitrile1:1.(Căpriță, *et al.*, 2007). The mycotoxin standards were supplied-by-Sigma-Aldrich.

4- Changes in some chemical, physical and rheological properties of wheat grains caused by some mold fungi:

a- Preparation of whole flour:

Contaminated and un-contaminated wheat grain samples were milled using a laboratory disc mill (Quadrumat junior flour mill, Model Number : 179510, Type: 279002, Brabender, OHG; Duisbarg ,Germany) for obtaining whole wheat flour in Food Technology Dept, National Research Centre, Dokki, Cairo, Egypt

b-Chemical Composition:

The samples were analyzed for moisture, protein, fat, crude fiber, wet and dry gluten contents, according to standard methods (AACC, 2000).

c-Rheological propertes of whole wheat flour dough:

Rheological properties of whole wheat flour dough were tested using Farinograph (300g testing bowl), Model No :178507, Type: 8100122, (31, 50 and 63 rpm) Brabender ,OHG ,Germany and Extensograph, Model No 179516,Type : 86000, Brabender, OHG, Germany ,thre pieces of dough were prepared to Extensograph tests according to (AACC., 2000).

d- Color quality of whole wheat flour:

Control and contaminated samples were measured using a spectrocolorimeter with the CIE color scale (Hunter, Lab scan XE). This instrument was standardized against the white tile of Hunter Lab Color standard (Lx No, 16379): X= 77.26 , Y= 81.94 and Z= 88.14.The L ,a and b values were reported (AACC., 2000).Total color difference ($\triangle E$) was calculated as:

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$$\triangle E = [(\triangle L)^2 + (\triangle a)^2 + (\triangle b)^2]^{1/2}$$

5- Decontamination of fungi:

Using some physical and chemical prevention means to reduce the mycological pollution of grain surface according to (János, *et. al.,* 2010).

a- Ozone as physical decontamination treatment:

Ozone delivered from a generator on site upon demand, at the concentration 3% by weight. The exposure of dry and imbibed seeds on ozone was for 3 minutes, to make a efficient disinfection. (Ciccarese, *et. al.,* 2007).

b- Hot air were used at 55°C. for 5 min.

c- Sodium hypochlorite as chemical decontamination treatment:

d- Test of germination:

Germination was tested by making treated and untreated seeds on Petri dishes (90mm diameter) under an imbibed filter paper in the dark and at the temperature of 26°C. Germination was recorded during one week. Finally the percentage of germinated seeds was calculated**.** Fungi development test untreated and treated seeds (dry and imbibed) were incubated on Petri dishes in PDA of substrate with antibiotics, at the temperature of 26°C to stimulate the development of saprophytes fungi. Colonies of fungi were controlled and identified. The percentages of seeds contaminated with each fungi and fungal flora were calculated (Gonzalez *et. al*., 1999, Ciccarese, *et. al.,* 2007 and Fatma Bensassi, *et. al*., 2011).

6- Statistical analyses:

The data were subjected to analysis of variance and Duncan's multiple rang test was used to differentiate means at 5% (Duncan, 1955).

Results and Discussion

1-Mycoflora associated wheat grain samples:

Isolation of the mycoflora contaminated wheat grain samples which collected from four different Governorates, in Egypt Qalubya, Dekahlya, Gharbya and Menufya by using two tested standard methods namely agar plate and blotter test were recorded in Table (1). Data in this table presented that, agar plate (PDA medium) gave higher values and colony percent of fungi associated with wheat grain in all tested governorate samples than blotter test. Also, data in this table show that, disinfections of the tested wheat grains lead to higher percentage of germinated seeds in both agar plate method and blotter test comparing with non-disinfected grains. On the other hand, the infection percent was higher with non-disinfected seeds compared with disinfected seeds. These results were finding by (Mathur, *et. al*.,2003).

Table 1: Germination and infection percent of some disinfected and non disinfected wheat grain samples which collected from different governorates by using blotter test and agar plate methods.

	Blotter test								Agar plate (PDA)							
	Disinfected				Non disinfected				Disinfected				Non disinfected			
Governorates	T.G	%	T.C	%	T.G	%	T.C	%	T.G	%	T.C	%	T.G	%	T.C	%
Qalubya	58	96.7	14	23.3	52	86.7	34	56.7	59	98.3	29	48.3	48	80.0	47	78.3
Dekahlya	60	100.0	16	26.7	54	90.0	36	60.0	49	81.7	46	76.7	40	66.7	56	93.3
Gharbya	57	95.0	6	10.0	53	88.3	24	40.0	57	95.0	40	66.7	46	76.7	54	90.0
Menufya	60	100.0	10	16.7	54	90.0	32	53.3	54	90.0	34	56.7	47	78.3	55	91.7
Total	235	97.9	46	19.2	213	88.8	126	52.5	219	91.3	149	62.1	181	75.4	212	88.3

T.C = Total fungal colonies, T. G = Total of germinated grains
PDA = Potato dextrose agar

2- Fungal frequency associated with wheat grain samples:

Isolation from tested wheat grain samples which collected from different Governorates yielded 460 fungal isolates presented in Table (2). Data show that, agar plate (PDA) was enhanced test and yielded 270 fungal

isolates (58.7%) while blotter method gave 190 isolates only (41.3%). These results are in agreement with Mathur, *et. al*., (2003). The same table indicated that, eight fungal genera were isolated and identified as: *Alternaria, Aspergillus (A. niger, A. flavus* and *A. parasiticus), Drechslera sp, Epicoccum purpurascenes, Fusarium moniliform, Mucor* sp., *Penicillium* sp. and *Rhizopus stolonifer*. Also data presented that, *Alternaria (A. tenuis* and *A. alternata)* was the most fungal frequency occurred and isolated from all tested wheat grain samples in all different Governorates which recorded 170 isolates (36.9 %) followed by *Rhizopus stolonifer* 115 isolates (25.0 %). *Mucor* sp. was less fungal frequency and gave (1 isolate) with 0.2%. On the other hand, Dekahlya wheat grain sample was the most fungal frequency than other governorates which gave 144 isolates (31.3 %) followed by Qalubya, and Menufya wheat grain samples which recorded (27.2 and 20.9 % respectively), but Gharbya sample gave less fungal frequency which recorded 95 isolates (20.6%). Similar results were obtained by (Muthomi & Mutitu, 2003, Aslam 2005 and Maliha, *et. al*., 2010.

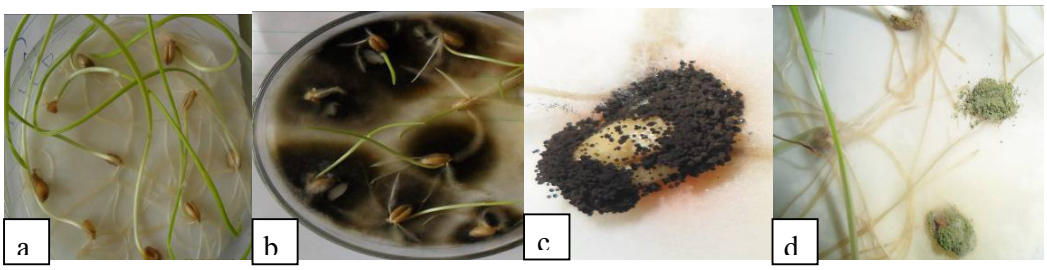

Fig (1): a-Healthy wheat grains, **b**- Contaminated with ***Alternaria,***
c- With ***Aspergillus niger*** **d**- With ***Aspergillus parasiticus***

Mycotoxin production:

Results of mycotoxins concentration produced by some toxigenic fungi which isolated from wheat grains were tabulated in Table (3). It is clear that two isolates of *A. flavus* (No.1&10) isolated from Qalubya and Gharbya samples, one isolate of *A. parasiticus* (No.54) from Menufya sample and one isolate of *Fusarium moniliforme* (No.19) isolated from Dekahlya grain sample had the ability to produce mycotoxins in significant concentrations. The total aflatoxins concentration was 100.49 µ/kg belong to 96.0 and 4.49 µ/kg of AFB_1 and AFB_2 , respectively in Qalubya sample, the total aflatoxins concentration was 145.77 µ/kg belong to 108.45 and 37.32 µ/kg of AFB_1 and AFB_2 , respectively in Gharbya sample, the total aflatoxins concentration was 127.82 µ/kg belong to 93.77, 23.15, 4.7 and 6.2 µ/kg of AFB_1, AFB_2, AFG_1 and AFG_2 , respectively in Menufya sample whereas, fumonisin concentration was 0.316 mg/kg in wheat grain Dakahlya sample. On the other hand, the same table show that no fumonisins were detected with Qalubya, Gharbya and Menufya grain samples. Also, no-aflatoxins were detected with Dakahlya sample The same results were reported by (Scussel, 2002; Scudamore, 2005 and Ciccarese, *et. al.,* 2007). They reported that, seed contamination by fungi may be dangerous either in the case in which seed is used in sowing either when it is stored as human or animal food. Superficial contamination of seed for human or animal feeding during storage and marketing derives from infections produced in field and from saprophytic fungi as *Aspergillus* spp. *Penicillium* spp and *Fusarium* spp. They produce dangerous metabolites for human or animal health (aflatoxin B1, B2, G1, G2, M1, ocratoxin A, zearalenone and fumonisin) (Ciccarese, *et. al.,* 2007).

4- Changes in chemical composition:

Chemical analyses of whole wheat flour were recorded in Table (4). Data in this table show that, moisture contents of all contaminated samples were in a narrow rang of 8.32- 9.01% and reached to 10.92 % for un-contaminated (control). Meanwhile, ash content was 1.68% with un-contaminated (control) sample, while reached to 2.71% for contaminated sample with *A. flavus*. Other contaminated samples displayed intermediate values being 1.78% for sample contaminated by *Fusariam graminearum* and 2.07% with *pencillium sp*. Protein contents of all contaminated samples were significantly higher than that of control. Contaminated sample by *A. flavus* ranked first (12.04%) followed by sample contaminated with *A. parasiticus* (10.66%), and sample contaminated with *Pencillium* and *Fusarium* (10.53 and 9.81%), respectively. Same findings were noticed when fat % was considered. However, the crude fiber and carbohydrates showed a reversible trend with the protein and fat of the contaminated samples. Data in the same table proved that wet and dry gluten of control reached 16.2 and 7.4 %, respectively. Meanwhile, all contaminated samples were not formed gluten . This may be due to damaged gluten fractions (gliadin and glutenin) by protolytic enzymes in highly infected wheat. Wheat kernels that were lightly and moderately infected by *Fusarium graminearum* were analyzed in terms of their

charbohydrate, lipid, and protein contents to determine any compositional changes (Boyacioglu and Hettiarachchy (2005). They reported that the significant compositional changes in lightly infected wheat were increase in reducing sugars (24%), no – starch lipids (5%) and decrease in cellulose (17%) and hemicellulose (20 %) components. In moderately infected wheat, the increase in protein (6 %), total sugars (26 %), reducing sugars (14 %), non – starch (20 %) and starch (8 %) lipids, and decrease in apparent and total amylase (11–20 %), cellulose (43 %) and hemicellulose (37 %) components were also significant . Furthermore, infection with F. graminearum decreased the proportions of water – extractable protein (albumin) and storage protein (glutenin) by 33 and 80 %, in moderately infected wheat, in comparison to the control sample. These results were finding by (Barabara, *et al .,* 2004, Jackowiak, *et al.,* 2005 and Chandra, *et. al* ., 2011).

Table 2: Fungal frequency occurred with some wheat grain samples using two standard methods of isolation collected from different governorates.

Test		Blotter test								Agar plate (PDA)								T.C
		Disinfected				Non disinfected				Disinfected				Non disinfected				
Species		Qalubya	Dekahlya	Gharbya	Menufya	Qalubya	Dekahlya	Gharbya	Menufya	Qalubya	Dekahlya	Gharbya	Menufya	Qalubya	Dekahlya	Gharbya	Menufya	
Alternaria spp	T.C	12	2	12	9	15	5	18	8	4	2	11	7	17	11	22	15	170
	%	2.6	0.4	2.6	2.0	3.3	1.1	4.0	1.7	0.9	0.4	2.4	1.5	3.7	2.4	4.8	3.3	36.9
Aspergillus flavus	T.C	0	1	0	0	0	1	0	0	1	3	0	1	0	5	0	0	12
	%	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.2	0.7	0.0	0.2	0.0	1.1	0.0	0.0	2.6
A. niger	T.C	0	5	0	0	2	3	0	0	0	9	0	1	2	10	2	7	41
	%	0.0	1.1	0.0	0.0	0.4	0.7	0.0	0.0	0.0	2.0	0.0	0.2	0.4	2.2	0.4	1.5	8.9
A.parasiticus	T.C	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	4
	%	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.2	0.9
Drechslera sp.	T.C	0	0	1	0	1	0	1	0	0	1	0	0	1	0	0	1	6
	%	0.0	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.2	1.3
Epicoccum purpurascenes	T.C	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	3
	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.0	0.0	0.7
F. moniliforme	T.C	2	0	0	8	2	0	2	8	0	1	0	0	0	0	0	1	24
	%	0.4	0.0	0.0	1.7	0.4	0.0	0.4	1.7	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	5.2
Mucor sp.	T.C	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Penicillium sp.	T.C	2	17	0	0	0	17	0	2	2	19	3	5	1	14	1	1	84
	%	0.4	3.7	0.0	0.0	0.0	3.7	0.0	0.4	0.4	4.1	0.7	1.1	0.2	3.0	0.2	0.2	18.3
Rhizopus stolonifer	T.C	2	0	0	0	30	3	0	0	8	8	10	3	18	6	11	16	115
	%	0.4	0.0	0.0	0.0	6.5	0.7	0.0	0.0	1.7	1.7	2.2	0.7	4.0	1.3	2.4	3.5	25.0
T.C		19	25	13	17	50	29	21	19	16	43	25	18	40	47	36	42	460
%		4.1	5.4	2.8	3.7	11.0	6.3	4.6	4.1	3.5	9.3	5.4	4.0	8.7	10.2	7.8	9.1	100.0

T.C = Total Colonies

Table 3: Mycotoxin production.

Governorates	Aflatoxins (µ/kg)					Fumonisin
	AFB_1	AFB_2	AFG_1	AFG_2	Total	FB_1
Qalubya	96.0	4.49	ND	ND	100.49	ND
Dakahlya	ND	ND	ND	ND	000.00	0.316
Gharbya	108.45	37.32	ND	ND	145.77	ND
Menufya	93.77	23.15	4.7	6.2	127.82	ND

ND = Not detected

5- Color quality:

Color quality was recorded in Table (5). Data presented that, contaminated samples were darker in color compared with control. The lightness "L" values of contaminated samples were in a narrow range 47.03 – 61.02 but 78.20 for control. This lightness reduction was more pronounced in sample contaminated by *Pencillium* . Comparison among "a" values (degree of redness) of samples indicated that sample contaminated with *A. parasiticus* was clearly redder as compared to the control or other contaminated samples. The higher "a" values of contaminated samples may be attributed to the presence of the red to brown pigments naturally produced by fungus. Values of "b" (degree of yellowness) of samples ranged from 11.79 to 16.64 . Again, sample , tended

to have lower " b " values indicating lower degree of yellowness than those of control sample. The reduction in the degree of yellowness was minimum in case of sample contaminated with *Pencillium*. The highest color differences " $\triangle$E " 31.21 were recorded for samples contaminated with *Pencillium* followed by *A. flavus*. These results confirmed those obtained by (Dexter, *et al*., 1996), they reported that red spring wheat contaminated with *Fusarium* resulted in altered flour color.

Table 4: Changes in chemical composition of whole wheat flour (% on dry weight basis)

		Sample				
		Control	Sample contaminated by			
			A. parasiticus	*Pencillium sp.*	*Fusarium sp.*	*A. flavus*
Moisture		10.92	8.50	8.40	9.01	8.32
Ash		1.68	1.95	2.07	1.78	2.71
Crude protein		9.74	10.66	10.53	10.20	12.04
Fat		2.60	2.76	2.96	2.95	3.00
Crude fiber		3.55	2.40	2.26	2.80	2.58
* CHO		82.43	82.23	82.18	82.27	79.67
Gluten	Wet	16.2	-	-	-	-
	Dry	7.4	-	-	-	-

* Total carbohydrates was calculated by difference . Data are average of triplicate analysis.

Table 5: Color quality of whole wheat flour of contaminated samples

	Sample				
	Control	Sample contaminated by			
		A.. parasiticus	*Pencillium sp*	*Fusarium sp*	*A.. flavous*
Lightness "L"	78.20	50.63	47.03	61.02	48.76
Redness "a"	2.61	6.64	3.28	4.72	5.02
Yellowness "b"	13.20	15.97	11.79	16.64	13.82
$\triangle$E	0.00	28.00	31.21	17.64	29.54

6- Rheological properties of whole wheat flour dough:

The effect of infestation of wheat grains by tested fungi on the farinograph and extensograph parameters was reported in Table (6). Results showed that control sample had 68.5%, 5.0 min, 6.0 min, 80 BU and 100 BU for water absorption, dough development time, dough stability, mixing tolerance index and dough weakening , respectively. Also, control sample had 130 mm, 300 BU, 2.0 and 70 Cm2 for dough extensibility, resistance to extension, proportional number and dough energy, respectively. These results are in agreement with those obtained by (Alian, *et al* 1997), they reported that wholmeal wheat doughs had higher water absorption , mixing time and dough weakening and lower dough stability compared to those of wheat flour 72 % extraction. They also motioned that wholemeal doughs had lower extensibility, resistance to extension and dough energy than those of wheat flour.

The dough structure was not formed for all highly contaminated samples. Consequently these samples didn't give farinograms and extensograms. The undesirable effect on the rheological properties of contaminated dough may be due to breakdown of dough proteins and starch as a result of the action of protolytic and amylolytic enzymes. The strength of dough depends on the actual nember of protein or gluten particles per unit of dough, and secondly on the efficiency of protein particles to swell by hydration . Other possible explanation is that the presence of fungus weakened the dough quality because of lacking the gluteneous complex as does those of control sample. The previous results were further supported by determine the wet and dry gluten. Fungal amylolytic decomposition of Fusarious wheat kernel leads to degradation of small starch granules (Jackowiak, *et. al.*, 2005), and due to decrease in total specific area of starch granules and higher degree of damage of starch component of endosperm , in comparison to healthy kernels , Fusarious kernel becomes more subjected to decomposition of starch component by fungal alpha- amylase. The consequence of this phenomenon is the decrease in amylograph peak viscosity.

Elizabet, *et al.,* (2011), reported that increase of content of Fusarious kernels is accompanied with trends of decrease in Farinograph quality number, dough stability, and resistance, and trends of increase in dough degree of softening, Extensograph energy, resistance and ratio number.Stitistically significant differences were confirmed only in the case of Amylograph peak viscosity without confirmatation based on falling number value , but in accordance with the findings of some authors, (Wang *et al.,* 2005), who discovered that the samples with extremely high content of Fusarious kernels were characterized with abviously higher values of hagberg falling number, whereas, sacchorse content in flour decreased. Other authors stated that contamination of wheat with storage molds (*Aspergillus* spp. and *penicillium* spp) resulted in decrease of baking performance of wheat flour.

Table 6: Farinograph and Extensograph parameters of whole wheat flour dough .

Parameters	Samples				
	Control	Contaminated samples with four tested fungi			
		A.. parasiticus	*Pencillium sp.*	*Fusarium sp.*	*A.. flavous*
Farinograph parameters : Water absorption (%)	68.5	-	-	-	-
Dough development time (min)	5.0	-	-	-	-
Dough stability (min)	6.0	-	-	-	-
Mixing tolerance index (BU)	80.0	-	-	-	-
Dough weakening (BU)	100.0	-	-	-	-
Extensograph parameters : Extensibility "E" (mm)	130.0	-	-	-	-
Resistance to extension "R" (BU)	300.0	-	-	-	-
Proportional number (R/E)	2.0	-	-	-	-
Dough energy (Cm^2)	70.0	-	-	-	-

BU = Brabender unit

* The samples didn't gave farinograms and extensograms.

7- Decontamination of fungi:

Effect of some physical and chemical methods on percentage of fungal colonies associated wheat grain samples using blotter test were recorded in Table (7). Data presented that, all treatments (both physical and chemical) were found to be decreased significantly total fungal colonies (infected wheat grains) than untreated (control). Also data in this table presented that, chemical treatment (by sodium hypochlorite) was more effective than physical methods with all treated samples. Ozone treatment was enhanced than hot air treatment. Data also show that, decreased infection percent (total fungal colonies) from 46.7% with untreated wheat grain of Qalubya sample to 30.0, 10.0% and zero percent when treated by hot air, ozone and sodium hypochlorite respectively and from 46.7% with untreated Dakahlya sample to 40.0% with hot air and sodium hypochlorite treatments to 30.0% with grains treated by ozone. Also, decreased infection percent (total fungal colonies) from 50.0% with untreated wheat grain of Gharbya sample to 6.7% with hot air and zero percent when treated with ozone and sodium hypochlorite respectively and from 40.0% with untreated Menufya sample decreased to 36.7% with hot air, 26.7% when treated with ozone and 6.7% with sodium hypochlorite. Similar results were recorded by (János, *et. al.,* 2010). Also, Brandon Jeong, (2010) repotted that, ozone was a strong oxidant effective in controlling bacteria, molds, protozoa, and viruses. Ozone decays more than 99.99% of fungus, moss, and bacteria within 10 seconds. Ozone can be used for disinfection, cleansing, and deodorization without side effects. It was initially used as an alternative disinfectant to hypochlorite. Ozone might have different applications, such as cleaning surfaces or equipment and disinfecting water for recycling. Continuous exposure to low concentrations in storage areas can oxidize ethylene, and treatments with ozone gas have been shown to elicit the accumulation of antioxidants. Because the lack of residues on the product. Palou, L. *et. al.,* (2001 & 2002) motioned that, ozone can be used as a relatively brief pre-storage or storage treatment in air or water, or as a continuous or intermittent component of the atmosphere throughout storage or transportation.

The direct effect of heat treatments on reducing the inoculum size and minimizing the rots includes slowing germ tube elongation or inactivation and outright killing of germinating fungal spores (Karabulut, 2005). Exposing fungal spores of *A. alternata* and *Fusarium solan*i to 60°C for about 15 s *in vitro* resulted in 48 and 42% reduction in spore germination, respectively (Fallik *et al*., 2000). *In vitro* inhibition of *Alternaria alternata* and *Fusarium solani* with increasing times of exposure.

Table 7: Effect of physical & chemical treatments on percentage of fungal colonies associated with wheat grain samples

Treatment		Qalubya	Dekahlya	Gharbya	Menufya	Mean
Physical	ozone	10.0 d	30.0 bc	0.0 d	26.7 c	16.67 C
	Hot air	30.0 bc	40.0 abc	6.7 d	36.7 abc	28.33 B
Chemical Sodium hypochlorite		0.0 d	40.0 abc	0.0 d	6.7 d	11.67 C
Control		46.7 ab	46.7 ab	50.0 a	40.0 abc	45.83 A
Mean		21.67 BC	39.17 A	14.17 C	27.50 B	

8-Inhanced of germination percent:

Effect of physical & chemical treatments on germination percent of wheat grains tabulated in Table (8) and Fig (2). Data in this table show that, both physical and chemical treatments were found to increased

germination percent of all wheat grain samples (collected from different localities). Ozone enhanced treatment than others. No significant between treatments. Different was found in between Governorates. On the other hand the same table presented that, Physical treatments i.e. ozone and hot air increased germination percent from 90% with untreated grains to hundred percent of germination seeds with Qalubya sample followed by chemical treatment (sodium hypochlorite) which recorded 96.7%. In Dakahlya sample. Germination percent reached from 20% with untreated (control) to 26.7% with hot air treatment and 70% with ozone treatment. In Gharbya: Increasing germination percentage of wheat grain sample from 86.7% with untreated grains to 96.7% with ozone and sodium hypochlorite reached to hundred percent with hot air treatment. Finally, increasing germination percentage of wheat grains (Menufya Governorate) from 76.7% with untreated sample to 76.7% with hot air, 86.7% with ozone treatment reached to 90.0% with sodium hypochlorite. These results were obtained by (Palou, L. *et. al.,* 2001 & 2002, János, *et. al.,* 2010 and Brandon Jeong, 2010.

Table 8: Effect of physical & chemical treatments on germination percent of wheat grains

Treatment		Qalubya	Dekahlya	Gharbya	Menufya	Mean
Physical	ozone	100.0 a	70.0 c	96.7 a	86.7 ab	88.33 A
	Hot air	100.0 a	26.7 d	100.0 a	76.7 bc	75.83 B
Chemical Sodium hypochlorite		96.7 a	20.0 d	96.7 a	90.0 a	75.83 B
Control		90.0 a	20.0 d	86.7 ab	76.7 bc	68.33 C
Mean		96.67 A	34.17 C	95.0 A	82.5 B	

Fig. 2: Decontamination of fungi.

Conclusion:

Among various factors that affect seed health, the most important are the seed borne fungi that not only lower seed germination, but also reduce seed vigor resulting in low yield. Healthy seed plays an important role not only for successful cultivation but also for increasing yield of crop**.** These pathogens may affect the plant resulting in a reduction of the grain quality; some species can even release natural toxins that cause human and animal intoxication. Several seed-borne pathogens are known to be associated with wheat seed which are responsible for deteriorating seed quality during storage**.** Highly contamination of wheat kernels with storage moulds resulted in altered flour color, changes in chemical composition, deterioration in dough rheological properties, and making the wheat unsuitable for bread making.

There are several physical methods of decontamination of agricultural products known to us such as the removal of damaged grains or of a part of contaminated crop, washing procedures, ozonation. Heat treatment (hot air technology), are relatively simple techniques, economical, safe from rapid temperature changes and applicable to many potential postharvest problems. These treatments have also been very effective in controlling

fungi. Chemical compounds such as alkaline compounds (sodium hydroxyde) has efficacy in decontamination of toxigenic fungi.

References

AACC., 2000. Approved Methods of the American Association of Cereal Chemists, *10th edn. St. Paul, Minnesota , USA.*

Alian, A.M., A. Ramy Abdel-latif, A.A.E. Yaseen and A.A. Shouk, 1997. Chemical and biological evaluation of whole meal wheat bread . *Egypt. J. Food Sci.,* 25(1): 121-138.

A.O.A.C., 2005. Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International 17th ed., Nature Toxins. AOAC International, Arlington, Virginia, USA, chapter 49.

Aslam, R.M., A. Mumtaz, L. Mubeen, S. Sarwar and A.K. Khalil, 2005. Studies on Seed-Born Fungi of Wheat in Sindh Province and Their Effect on Seed Germination Pak J. Bot., 37(1): 181-185.

Attitalla, H.I., K.T. Al-Ani, N.A. Laith, Muftah, A.A. Balal, Z.M. Ibrahim, S.M.S. El-Maraghy and K. Rezaul, 2010. Screening of fungi Associated With Commercial Grains and Animal Feeds in Al-Bayda Governorate, Lybia. *World Applied Science Journal.,* 9(7): 746-756.

Barabara, K., T. Mona, S. Estein and A. Birgitte, 2004. Alternaria and Fusarium in Norwegain grains of reduced quality –a matched pair sample study . *International Journal of Food Microbiology,* 93(1): 51-62.

Barnett, H.L. and B.B. Hunter, 1977. Illustrated genera of imperfect fungi, 3rd Ed. *Burgess Publishing Company, Minnesota.* pp: 2412.

Boyacioglu, D. and N.S. Hettiarachchy, 2005. Changes in some biochemical components of wheat grain that was infected with *Fusarium graminearam* . *Journal of Cereal Science,* 21(1): 57-62 .

Brandon Jeong, 2010. Premium Care Appliance Most Advanced Hygienic System: Eco9 Sterile Water Generator E-mail. freebdj@gmail.com

Căpriță, A., Rodica Căpriță; C. Cozmiuc, M. Bianca and H. Sărăndan, 2007. Simultaneous Determination of Mycotoxins (Ochratoxin A and Deoxynivalenol in Biological Samples. Journal of Agro alimentary Processes and Technologies, Volume XIII, 2: 353-358.

Chandra, B.S., S.J. Digvir, B. Ferenc and D.C.W. Noel, 2011. Synchrotron based infrared study of compositional changes in stored wheat due to infection with *Aspergillus glaucus* . Jounal of Stored Products Research, 47(4): 372-377.

Ciccarese, F.N., A. Sasanelli, T. Ciccarese, A. Ziadi, A. Ambrico and L. Mancini, 2007. Seed disinfestation by ozone treatments. *IOA Conference and Exhibition Valencia, Spain* - October 29-31.

Dexter, J.E., R.M. Clear, and K.R. Preston, 1996. Fusarium head blight: effect on the milling and baking of some Canadian wheats. *Cereal Chemistry,* 73(6): 695-701.

Doohan, F.M., J. Brennan and B.M. Cooke, 2003. Influence of climatic factors on *Fusarium* species pathogenic to cereals. Eur. *J. Plant Pathol.,* 109: 755-768.

Duncan, D.B., 1955. Multiple range and multiple "F" tests. Biometrics, 11: 1-42.

Elizabet, P.J.H., M. Aleksandra, J. Torbica and S. Mastilovic, 2011. Influence of degree of wheat infestation with Fusarium on its technological quality and safety. Proc. Nat. Sci, Matica Srpska Novi Sad, 120: 61-70.

Fallik, E., 2004. Prestorage hot water treatments (immersion, rinsing and brushing). *Postharvest Biology and Technology,* 32: 125-134.

Fallik, E., Y. Aharoni, A. Copel, R. Rodov, S. Tuvia-Alkalai, B. Horev, O. Yekutieli, A. Wiseblum and R. Regev, 2000. A short hot water rinse reduces postharvest losses of 'Galia' melon. *Plant Pathology,* 49: 333-338.

Fatma Bensassi1, Chennaoui, M., B. Hassen and R.H. Mohamed, 2011. Survey of the mycobiota of freshly harvested wheat grains in the main production areas of Tunisia. African Journal of Food Science, 5(5): 292- 298.

Garcia, M.J. de M.A.M. Biaggioni, W.A. Ferreira, E.Y. Kohara and A.M. De Almeida, 2003. Sucessão de espécies de fungos em milho armazenado em sistema aerado. *Revista Brasileira de Armazenagem.,* 27: 14-22.

Gonzalez, H.H.L., E.J. Martinez, A. Pacin and S.L. Resnik, 1999. Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxynivalenol occurrence on Argentinian durum. Mycopathologia., 144: 97-102.

Jackowiak, H., D. Packa, M. Wiwart and J . Perkowski, 2005. Scanning electron microscopy of Fusarium damaged kernels of spring wheat. International Journal of Food Microbiology, 98(2): 113-123.

János, V., K. Sándor, P. Zsanett, V. Csaba and T. Beáta, 2010. Chemical, Physical and Biological Approaches to Prevent Ochratoxin Induced Toxicoses in Humans and Animals. *Toxins*, *2*, 1718-1750; doi:10.3390/toxins2071718.

Karabulut, O.A., G. Kuruoglu, K. Ilhan, U. Arslan, 2005. The use of heat treatments against postharvest diseases. Ondokuz Mays Universitesi, Ziraat Fakultesi Dergisi. 20(1): 94-101. many ref.

Maliha, R., K. Samina and A. Najma, 2010. Assessment of mycoflora and aflatoxin contamination of stored wheat grains. International Food Research Journal, 17: 71-81.

Maren, A.K. and I.P. Johan, 1988. A Laboratory guid to the common *Aspergillus* spp. and their teleomprph. Commnwealth Scientific and Industrial, pp: 116.

Marija, H., K. Tomislav, S. Drago, M. Marija and B. Snjezana, 2004. Fungal contamination of cooking and the raw materials for their production in Croatia. Czech Journal of Food Science, 22: 95-98.

Mathur, S.B and Olga Kongsdul, 2003. Common Laboratory seed healthy testing methods for detecting Fungi ,Danish Government Institute of Seed Pathology for Developing Countries Thorvaldsensvey57, DK-1871 Frederiksberg C, Copenhagen, Denmark. pp: 399.

Muthomi, J.W., 2001. Comparative studies on virulence, genetic variability and mycotoxin production among isolates of *Fusarium* species infecting wheat. Ph.D. Thesis, University of Nairobi.

Muthomi, J.W. and E.W. Mutitu, 2003. Occurrence of mycotoxin producing *Fusarium* species and other fungi on wheat kernels harvested in selected districts of Kenya. African Crop Science Conference Proceedings, 6: 335-339.

Nelson, P.E., T.A. Toussoun and W.F.O. Marassas, 1983. *Fusarium* species: An illustrated manual for identification. Pennsylvania State University Press, University park. pp: 193.

Ogido, R., C.A.F. Oliveira, D.R. Ledoux, G.E. Rottinghaus, B. Correa, T.A. Reis, E.G. Cules and lboquerque, R.A., 2004. Effects of prolonged administration of aflatoxin B1 and Fuminsin B1 in Laying Japanese quail. *Poull.sci.* 83: 1953-1958.

Palou, L., C.H. Crisosto, J.L. Smilanick, J.E. Adaskaveg and J.P. Zoffoli, 2002. Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. Post-harvest Biology and Technology, 24: 39-48.

Palou, L., J.L. Smilanick, C.H. Crisosto and M. Mansour, 2001. Effect of gaseous ozone exposure on the development of green and blue molds on cold stored citrus fruit. *Plant Diseases/* 85(6): 632-638.

Pryor, and Rice, 1999. Introduction to the use of ozone in food processing applications, In proceedings of the 14 th ozone World Congress. Dearborn, MI, USA. 22-26 August. *Pan American group, CT.* pp: 28-36.

Santos, J.P., 2002. Métodos preventivos de controle de pragas de grãos armazenados In: Lorini, I., Miike, L.H., Scussel, M.V. (Eds), *Armazenagem de grãos.Campinas, IBG,* pp: 399-441.

Scudamore, K.A., 2005. Identifying Mycotoxins is Paramount in the fight against their spread. Word Grain 23: 36-39.

Scussel, V.M., 2002. Fungus micotoxinas associados a grãos armazenados. Fungal and mycotoxins associated to storage of grains. In: Armazenamento de Grãos. Storage of Grains. Lorini, I., Scussel, V.M. and Miike, H.L. (Eds.) São Paulo: Ed. Chap. 9.3, IBG, 675-787.

Singh, K., C. Jenz, U. Thron and S.B. Mathur, 1991. An Illustrated of some Seed-born Aspergilli, Fusaria, Penicillia and their mycotoxins. First edition, Danish Government Institute of Seed Pathology for Developing Countries. Ryvangs Alle 78. DK-2900 Hellerup, Denmark and Department of Biotechnology.

Vicente, A.R., G.A. Martinez, P.M. Civello and A.R. Chaves, 2002. Quality of heat treated strawberry fruit during refrigerated storage. *Postharvest Biology and Technology,* 25: 59-71.

Wang, J., E. Pawelzik, J. Weinert and G.A. Wolf, 2005. Impact of *Fusarium culmorum* on the polysaccharides of wheat flour. Jounal Agricultural Food Chemistry, (14): 5818-5823.

Wang, Q.M., J.S. Wang, F.G. Yu, X. Zhu, K. Zaleta-Rivera and L. Du, 2006. Mycotoxin fumonisins: Health impacts and biosynthetic mechanism. Progress in Natural Science, 16(1): 7-15.

Whangchai, K., K. Saengnil and J. Uthaibutra, 2006. Effect of ozone in combination with some organic acids on the control of post-harvest decay and per-carp browning of long an fruit. Crop Prot. 25: 821-825.

Wiese, M.V., 1984. Compendium of wheat diseases. 3rd Ed. The American Phytopathological Society, pp: 106.