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Determination of Several Antioxidants Parameters and Deoxynivalenol in Iraqi and Other Types of Wheat

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Abstract: Fusarium species contaminate cereal crops international and sources the important diseases Fusarium head blight and crown rot in wheat. Fusarium pathogens decrease yield and some types produce trichothecene mycotoxins, like deoxynivalenol (DON), through the infection. These toxins play roles in pathogenesis on wheat and have serious health effects if present in grain consumed by humans or animals. The present study aimed to evaluate the extent of mycotoxins contamination and their occurrence by measuring the deoxynivalenol (DON) level, antioxidant enzymes like catalase(CAT), ascorbate peroxidase (APX) and non-enzymatic antioxidant such as beta carotene in Iraqi and other types of wheat grain before and after three months storage according to types in different stores. The results showed that deoxnivalenol, super oxides radical and hydrogen peroxide (H_2O_2) levels were significantly increase before and after three months storage according types in all stores, while catalase(CAT), ascorbate peroxidase (APX) showed a significant decrease. The present study conclude that activity of antioxidant enzymes such as catalase(CAT), ascorbate peroxidase (APX) were low, super oxides radical and hydrogen peroxide (H_2O_2) content were increase, the two reacting together to produce hydroxyl radical. This radical attacks all biomolecules and disturbs cell metabolism.The reduction in all type of antioxidant (enzymatic and non –enzymatic) result in increase in free radical and speed up in wheat damage may be due to the contamination of wheat with DON and exclusively being the form of a nutrition item when the chief components of the people in Iraqi.

Key words: Wheat · Deoxynivalenol · Catalase · Ascorbate peroxidase · Beta carotene

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is major crop plants of the grass family *Poaceae* (*Gramineae*), wheat is the leading food grain crop of the world and the most plenteous food crop, based on the area planted and in fact it is competing with the rice in the area harvested. It is the most important food of about two billion people (36% of the world population) and the world's most important cereal crop in terms of both area cultivated and amount of grain produced [1-3]. Deoxnivalenol natural occurring mycotoxins also referred to as vomitoxin. It is widely spread cereal pathogen can produce by secondary fungal type *B trichothecenes,* Trichothecenes are fungal secondary metabolites the animal and human toxicities of which have been extensively studied *Fusarium* graminearum, Wet and cool weather from flowering time to maturity promotes infection resulting in scab or head blight in wheat the infected scab has a tendency to have lighter weight kernels [4,5]. Reactive oxygen species are chemical species with an oxygen molecule containing one or more unpaired electrons include hydrogen peroxide which is generated naturally in chloroplasts during the light phase then diffuse out into the cytosol, plant cells respond defensively to oxidative stress by removing the ROS and maintaining antioxidant defense compounds at levels that reflect surrounding environmental conditions [6, 7]. Malondialdehyde (MDA) is one of many low molecular weight end-products of lipid hydoperoxide decomposition and it is the most often measured as an

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index of lipid peroxidation [8]. Plant have many enzymatic and non enzymatic defense such as catalase (EC: 1.11.1.6, oxidoreductase, CAT) is a tetramer iron porphyrin. It has highest turnover numbers of all enzymes, one catalase molecule can convert 40 million of molecules of hydrogen peroxide to water and oxygen each second [9]. Beta carotene isoprenoid compound is a lipophilic organic compounds located in the plastids that are distributed widely in plant. It is a non-enzymatic defense found in the chloroplasts and chromoplasts of plants [10]. Plants are naturally provided by enzymatic and non-enzymatic defense systems such as ascorbate peroxidases (EC: 1.11.1.11) belongs to the class I heme-peroxidases, it has a higher affinity for H2O2 is the main enzyme responsible for H2O2 removal in the chloroplast, peroxisomes and mitochondria [11,12]. Phenolic acids are antioxidant found in all higher plants the basic structural feature of phenolic compounds are an aromatic ring bearing one or more hydroxyl groups they high reactivity scavenging ROS playing as electron doner in the form of esters, glycosides or amides rarely found in free form [13].

MATERIALS AND METHODS

Experiment was conducted using wheat (*Triticum aestivum L.*) genotype Fareed taken from different places in Iraqi siloes and different species of [domestic and imported wheat] from harvesting of crop year 2013.

Total Phenolic Content (TPC): A micro colorimetric method was used for total phenolic assay, which utilizes Folin-Ciocalteu (F-C) reagent. For extraction of TPC, seeds 0.1 g were ground in 85% ethanol and kept at room temperature for 10 min then centrifuged at 15,000×*g* for 20 min at 4°C, the supernatant was separated and used for the determination of total phenolic content. For estimation of TPC in seed samples 100µl of supernatant was mixed with 100µl of 10% (v/v) F-C reagent, vortex thoroughly and then 800µl of 0.7 M Na2CO3 was added. Blank corrected absorbance of samples was measured at 650nm [14].

Malondialdehyde (MDA): 0.5 g of leaf samples were homogenized in five ml of distilled water. An equal volume of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid solution was added and the sample incubated at 95°C for 30 min. The reaction stopped by putting the reaction tubes in the ice bath. The samples then centrifuged at 10000×g for 30 min. The supernatant removed, absorption read at 532 nm and the amount of nonspecific absorption at 600 nm read and subtracted from this value. The amount of MDA present calculated from the extinction coefficient of 155 $mM^{-1}cm^{-1}$. Enzyme activity and MDA content of samples were recorded [15].

β-Carotene: β-carotene content was determined by the procedure of Mishra and Gupta (1995). The absorbance was measured at 440 nm [16].

Hydrogen Peroxide (H_2O_2): It was determined according to Velikova V. *et al*., (2000) [17].

Extraction of Antioxidant Enzymes: For extraction of enzymes, 0.1 gm of seeds were ground in 50mM cold phosphate buffer (pH 7.8) and centrifuged at 15,000×*g* for 20 min at 4°C. The supernatant was separated and used for the determination of different enzyme activities.

Catalase (CAT): For the estimation of catalase, seeds were homogenized in a standard composed of 50mM KP buffer, pH 7and 1mM hydrogen peroxide (H2O2) [18].

Ascorbate peroxidases activity was measured according to Yoshimura et al. (2000) by monitoring the rate of ascorbate oxidation at 290 nm (E=2.8$mM^{-1}cm^{-1}$) [19].

Deoxynivalenol was measured by using ELISA kit.

RESULTS AND DISCUSSION

Tables 1, 2 showed the mean and standard deviation of MDA [nmol/gm FW] before and after 3 months of storage according types and according to the stores. The result showed a significant increase which may be due to the increased in free radical with the storage period because of the influence of climatic condition, MDA is one of many low molecular weight naturally occurring end-products of lipid hydro peroxide decomposition and is the most often measured as an index of lipid peroxidation malonaldialdhyde [20].

Beta carotene content [ppm] before and after 3 months of storage showed a significant decrease ($p<0.05$) in different types of wheat and showed a significant decrease in according to the storage stores in all types of wheat, Tables 3 and 4. The β- carotene showed a decreased with the length of the storage period and high temperature due to the increased of the reactive oxygen species, under normal condition the antioxidant defense system against the reactive oxygen species but in case of highly increase of the ROS as in stress (climate condition) this result in the production of oxidative stress [21].

Table 1: The mean and standard deviation of MDA [nmol/gm FW] before storage and after 3 months storage according types in all stores

			Comparison of Significant	
MDA [nmol/gm FW]	Before storage	After 3 months Storage	*p* Value	Sig.
Local First Class Wheat	92.62±5.11	109.15±6.43	0.004	*p*< 0.01 (S)
Local Second Class Wheat	90.52±9.17	109.80±8.11	0.005	*p*< 0.01 (S)
Australian wheat Origin	101.68±11.50	117.19±6.41	0.005	*p*< 0.01 (S)
Turkish wheat Origin	106.92±15.87	115.17±5.98	0.048	*P*< 0.05 (S)
Russian wheat Origin	108.57±12.20	117.36±4.65	0.047	*p*< 0.05 (S)
Harmed Damaged) wheat	166.19±11.76	177.76±7.67	0.049	*P*< 0.05 (S)

Table 2: The mean and standard deviation of MDA [nmole/gm FW] before and after 3 months storage according stores in all type

			Comparison of Significant	
MDA [nmol/gm FW]	Before storage	After 3 months Storage	*p* Value	Sig
AL-Rusafa store	110.74±33.58	129.16±30.23	0.018	*p*< 0.05 (S)
Al- Doura store	118.08±30.23	128.68±29.93	0.048	*P*< 0.05 (S)
Al- Essaouira store	113.25±27.02	134.29±31.71	0.013	*p*< 0.05 (S)
Al-Taji store	113.77±24.60	122.91±31.25	0.038	*P*< 0.05 (S)
Salman pak store	106.01±34.20	131.23±30.25	0.024	*p*< 0.05 (S)
Al-Kut store	91.52±21.33	118.72±24.78	0.047	*p*< 0.05 (S)
Al- Nasiriya store	94.17±28.30	119.69±43.87	0.045	*p*< 0.05 (S)
Al- Basra store	100.25±27.11	123.43±28.74	0.032	*p*< 0.05 (S)

Table 3: The mean and standard deviation of β- carotene content [ppm] before storage and after 3 months storage according types in all stores.

			Comparison of Significant	
β- carotene [ppm]	Before storage	After 3 months Storage	*p* Value	Sig
Local First Class Wheat	3.54±0.20	1.34±0.44	0.047	*p*< 0.05 (S)
Local Second Class Wheat	3.54±0.23	1.34±0.34	0.044	*p*< 0.05 (S)
Australian wheat Origin	3.92±0.59	1.32±0.44	0.042	*p*< 0.05 (S)
Turkish wheat Origin	3.91±0.43	1.49±0.53	0.038	*p*< 0.05 (S)
Russian wheat Origin	3.77±0.54	1.50±0.56	0.034	*p*< 0.05 (S)
Harmed(Damaged) wheat	1.31±0.27	0.67±0.18	0.030	*p*< 0.05 (S)

Table 4: The mean and standard deviation of β- carotene content [ppm]before and after 3 months storage according stores in all type.

			Comparison of Significant	
β- carotene [ppm]	Before storage	After 3 months Storage	*p* Value	Sig
AL-Rusafa store	3.23±0.99	0.93±0.21	0.001	*p*< 0.01 (S)
Al- Doura store	3.29±1.15	1.89±0.51	0.021	*p*< 0.05 (S)
Al- Essaouira store	3.61±1.21	0.82±0.23	0.001	*p*< 0.01 (S)
Al-Taji store	3.44±1.18	1.47±0.47	0.004	*P*< 0.01 (S)
Salman pak store	2.87±0.88	0.86±0.15	0.001	*p*< 0.01 (S)
Al-Kut store	3.71±1.09	1.79±0.55	0.003	*p*< 0.01 (S)
Al- Nasiriya store	3.07±0.82	1.47±0.39	0.001	*p*< 0.05 (S)
Al- Basra store	3.39±1.08	1.37±0.42	0.002	*p*< 0.01 (S)

Table 5: The mean and standard deviation of CAT [U/gm] before and after 3 months storage according types in all stores

			Comparison of Significant	
CAT [U/gm]	Before storage	After 3 months Storage	*p* Value	Sig
Local First Class Wheat	9.05±0.42	6.74±0.90	0.041	*p*< 0.05 (S)
Local Second Class Wheat	8.88±0.61	7.32±0.99	0.047	*p*< 0.05 (S)
Australian wheat Origin	9.23±0.49	7.37±1.12	0.043	*p*< 0.05 (S)
Turkish wheat Origin	8.94±0.68	7.28±1.22	0.048	*p*< 0.05 (S)
Russian wheat Origin	9.27±0.60	7.24±1.32	0.044	*p*< 0.05 (S)
Harmed(Damaged) wheat	5.31±0.79	3.19±0.93	0.040	*p*< 0.05 (S)

Table 6: The mean and standard deviation of CAT [u/gm]before and after 3 months storage according stores in all type

			Comparison of Significant	
CAT [U/gm]	Before storage	After 3 months Storage	*p* Value	Sig
AL-Rusafa store	8.01±1.32	6.99±1.68	0.071	*p*>0.05 (NS)
Al- Doura store	8.43±1.61	6.32±1.99	0.041	*p*< 0.05 (S)
Al- Essaouira store	8.21±1.69	5.88±1.22	0.038	*p*< 0.05 (S)
Al-Taji store	8.32±1.58	5.78±1.52	0.048	*p*< 0.05 (S)
Salman pak store	8.66±1.01	5.00±1.29	0.030	*P*< 0.05 (S)
Al-Kut store	8.42±1.60	6.24±1.81	0.040	*P*< 0.05 (S)
Al- Nasiriya store	8.87±1.11	6.98±1.76	0.080	*p*>0.05 (NS)
Al- Basra store	8.63±1.61	7.92±0.99	0.097	*p*>0.05 (NS)

Table 7: The mean and standard deviation of H_2O_2 content [µmol/gm FW] before and after 3 months storage according types in all stores

			Comparison of Significant	
H_2O_2 content [µmol/gm FW]	Before storage	After 3 months Storage	*p* Value	Sig
Local First Class Wheat	5.19±0.70	7.77±0.46	0.043	*p*< 0.05 (S)
Local Second Class Wheat	5.56±0.62	7.73±0.58	0.044	*p*< 0.05 (S)
Australian wheat Origin	5.69±0.98	7.84±0.23	0.039	*p*< 0.05 (S)
Turkish wheat Origin	5.84±0.93	7.93±0.54	0.037	*p*< 0.05 (S)
Russian wheat Origin	5.66±1.11	7.85±0.47	0.042	*p*< 0.05 (S)
Harmed(Damaged) wheat	8.89±1.36	12.63±1.54	0.032	*p*< 0.05 (S)

Table 8: The mean and standard deviation of H_2O_2 content [µmol/gm FW] before and after 3 months storage according stores in all type

			Comparison of Significant	
H_2O_2 content [µmol/gm FW]	Before storage	After 3 months Storage	*p* Value	Sig
AL-Rusafa store	5.90±0.97	8.15±1.83	0.040	*p*< 0.05 (S)
Al- Doura store	5.62±0.87	8.32±1.93	0.030	*p*< 0.05 (S)
Al- Essaouira store	7.07±1.37	9.35±1.85	0.042	P<0.05 (S)
Al-Taji store	6.59±1.42	8.34±1.12	0.048	*P*< 0.05 (S)
Salman pak store	6.91±1,24	9.26±1.99	0.037	*p*< 0.05 (S)
Al-Kut store	6.37±0.94	9.19±1.55	0.029	*p*< 0.05 (S)
Al- Nasiriya store	5.84±0.96	8.01±1.10	0.033	*p*< 0.05 (S)
Al- Basra store	5.87±1.34	8.96±1.51	0.015	*p*< 0.05 (S)

Table 9: The mean and standard deviation of TPC [µgm/gm FW] before and after 3 months storage according types in all stores.

			Comparison of Significant	
TPC [µgm/gm FW]	Before storage	After 3 months Storage	*p* Value	Sig
Local First Class Wheat	1.06±0.36	0.74±0.06	0.047	*p*< 0.05 (S)
Local Second Class Wheat	1.24±0.29	0.74±0.05	0.044	*p*< 0.05 (S)
Australian wheat Origin	1.42±0.33	0.80±0.09	0.029	*p*< 0.05 (S)
Turkish wheat Origin	1.24±0.17	0.78±0.10	0.035	*p*< 0.05 (S)
Russian wheat Origin	1.18±0.12	0.73±0.07	0.030	*p*< 0.05 (S)
Harmed(Damaged) wheat	0.72±0.13	0.48±0.09	0.031	*p*< 0.05 (S)

Table 10: The mean and standard deviation of TPC [µgm/gm FW] before and after 3 months storage according stores in all type.

			Comparison of Significant	
TPC [µgm/gm FW]	Before storage	After 3 months Storage	*p* Value	Sig
AL-Rusafa store	1.19±0.25	0.70±0.13	0.036	*p*< 0.05 (S)
Al- Doura store	1.38±0.51	0.76±0.11	0.015	*p*< 0.05 (S)
Al- Essaouira store	1.18±0.22	0.61±0.12	0.000	P<0.01 (S)
Al-Taji store	1.10±0.33	0.75±0.12	0.028	*P*< 0.05 (S)
Salman pak store	1.09±0.21	0.61±0.14	0.001	*p*< 0.01 (S)
Al-Kut store	1.10±0.39	0.74±0.13	0.038	*p*< 0.05 (S)
Al- Nasiriya store	0.89±0.23	0.75±0.09	0.326	*p*>0.05 (NS)
Al- Basra store	0.99±0.09	0.78±0.14	0.013	*p*< 0.05 (S)

Table 11: The mean and standard deviation of APX [U/gm] before and after 3 months storage according types in all stores.

			Comparison of Significant	
APX [U/gm]	Before storage	After 3 months Storage	*p* Value	Sig
Local First Class Wheat	0.131±0.02	0.078±0.01	0.030	*p*< 0.05 (S)
Local Second Class Wheat	0.141±0.02	0.078±0.01	0.026	*p*< 0.05 (S)
Australian wheat Origin	0.158±0.03	0.082±0.06	0.011	*p*< 0.05 (S)
Turkish wheat Origin	0.163±0.02	0.080±0.01	0.030	*P*< 0.05 (S)
Russian wheat Origin	0.146±0.02	0.081±0.03	0.025	*p*< 0.05 (S)
Harmed(Damaged) wheat	0.055±0.01	0.037±0.01	0.045	*P*< 0.05 (S)

Table 12: The mean and standard deviation of APX [U/gm] before storage and after 3 months storage according stores in all type.

			Comparison of Significant	
APX [U/gm]	Before storage	After 3 months Storage	*p* Value	Sig
AL-Rusafa store	0.134±0.05	0.072±0.01	0.018	*p< 0.05 (S)*
Al- Doura store	0.128±0.04	0.072±0.01	0.012	*p*< 0.05 (S)
Al- Essaouira store	0.141±0.04	0.059±0.01	0.001	*p*< 0.01 (S)
Al-Taji store	0.132±0.05	0.079±0.02	0.024	*p*< 0.05 (S)
Salman pak store	0.149±0.05	0.064±0.02	0.012	*P*< 0.05 (S)
Al-Kut store	0.121±0.03	0.083±0.02	0.043	*P*< 0.05 (S)
Al- Nasiriya store	0.131±0.02	0.078±0.01	0.034	*p*< 0.05 (S)
Al- Basra store	0.128±0.03	0.081±0.03	0.025	*P*< 0.05 (S)

Table 13: The mean and standard deviation of DON [ppm] before and after 3 months storage according to types in all stores.

			Comparison of Significant	
DON [ppm]	Before storage	After 3 months Storage	*p* Value	Sig
Local First Class Wheat	1.17±0.17	2.50±0.13	0.029	*p*< 0.05 (S)
Local Second Class Wheat	1.55±0.07	2.00±0.55	0.036	*p*< 0.05 (S)
Australian wheat Origin	1.00±0.05	2.03±0.69	0.014	*p*< 0.05 (S)
Turkish wheat Origin	1.02±0.07	1.74±0.65	0.038	*P*< 0.05 (S)
Russian wheat Origin	1.06±0.18	1.79±0.58	0.045	*p*< 0.05 (S)
Harmed(Damaged) wheat	2.41±0.48	4.25±1.01	0.025	*P*< 0.05 (S)

Table 14: The mean and standard deviation of DON [ppm] before and after 3 months storage according stores in all type

			Comparison of Significant	
DON [ppm]	Before storage	After 3 months Storage	*p* Value	Sig
AL-Rusafa store	1.51±0.73	1.66±0.68	0.727	p> 0.05 (NS)
Al- Doura store	1.41±0.63	3.14±1.85	0.004	*p*< 0.01 (S)
Al- Essaouira store	1.41±0.72	2.18±0.99	0.043	*p*< 0.05 (S)
Al-Taji store	1.26±0.42	2.48±0.75	0.024	*p*< 0.05 (S)
Salman pak store	1.62±0.71	3.79±1.58	0.002	*P*< 0.01 (S)
Al-Kut store	1.25±0.43	2.90±0.55	0.008	*P*< 0.01 (S)
Al- Nasiriya store	1.24±0.40	1.25±0.55	0.911	*p*> 0.05 (NS)
Al- Basra store	1.25±0.40	3.22±1.66	0.018	*P*< 0.05 (S)

Tables 5, 6 showed the mean and standard deviation of CAT specific activity [U/gm] before and after 3 months of storage according types and according to the stores. The contemporary study showed a significant decrease (p<0.05) in the mean of CAT specific activity [U/gm] between different types of wheat and showed a significant decrease (p< 0.05) in the mean according to the storage stores in all types of wheat except Al- Nasiriya, Albasra and Al Rusafa stores (p> 0.05) [22, 23].

The present study have been included evaluation the H_2O_2 content [µmol/gm FW] before and after 3 months of storage according types and according to the stores, the Tables 7, 8 showed a significant increase (p<0.05) between different types of wheat and indicated a significant increase in the mean according to the storage stores in all types of wheat H_2O_2.

Tables 9, 10 showed the mean and standard deviation of TPC [µgm/gm FW] before and after 3 months of storage according types and according to the stores, the present study indicated a significant decrease (p<0.05) in the mean between different types of wheat and shows a significant decrease in the mean of TPC [µgm/gm FW] according to the storage stores in all types of wheat except Al- Nasiriya store (p> 0.05). Total phenolic and H_2O_2 content are significantly contribute to an antioxidant due to the possess an aromatic ring bearing one or more hydroxyl substituent's and total phenolic may be found in free state, conjugated with sugars or esters or polymerized [24, 25].

Ascorbate peroxidase (APX) before and after 3 months of storage according types and according to the stores indicated a significant decrease (p< 0.05) in different types of wheat and shows a significant decrease in according to the storage stores in all types of wheat, Tables 11 and 12. Ascorbate peroxides activity which is important component of antioxidant system that play a key role in eradicating hydrogen peroxide molecular [26].

Table 13 and 14 showed a significant increase in the mean of DON [ppm] according to the storage stores in all types of wheat except Al-Rusafa and Al-Nasiriya stores showed a non-significant differences (p>0.05) after 3 months of Harmed wheat showed a highly increased in DON levels after 3 months storage especially in Al- Doura, Al-Kut, Salman pak, Al- Nasiriya and Al- Basra stores AL-Rusafa and Al- Nasiriya stores showed the low levels of DON in the all types of wheat except harmed wheat, these result may be due to separate the harmed wheat in isolated places and far from other types of wheat compared to the other stores. We showed to these results in previous study [27]. The increased in DON levels in wheat also due to the different climate condition formed in the field (and during storage) under favorable environmental and agronomical conditions such as warm and moist during fungal infection (grain flowering), moreover climate change will lead to shifts in the occurrence of mycotoxins in cereal grains [28, 29].

The present study conclude that membranes are one the major sites of cells and organelles damages. This is due to ROS can react through unsaturated fatty acids to cause peroxidation of necessary membrane lipids in intracellular organelles which leads to the escape of cellular substances, fast dryness and cell death and the reduction in all type of antioxidant cause the increase in free radical and speed up in wheat harm may possibly be due to the contamination of wheat with DON and absolutely being the form of a nutrition item when the chief components of the people in Iraqi.

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