Research Article

Influence of Water Activity and Time Duration on *Fusarium* Mycotoxins Production in Maize Grains During Post-Harvest Storage

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Abstract | This study investigated the influence of water activity (a_w) and storage time on total fungal and *Fusarium* counts and mycotoxins zearalenone (ZON), trichothecenes type-A (i.e. T-2 and HT-2) and trichothecenes type-B (i.e. DON, 3-ac DON, 15-ac DON) production in maize grains. Water activity of the grains was adjusted to 0.85, 0.90 and 0.95 a_w and the samples were stored at 25°C for 45 days. The control samples were kept without any a_w modification. At 15 days interval, the samples were analyzed for the aforesaid parameters. The data showed that a_w and storage time alone and interactively significantly ($p<0.05$) affected the analyzed parameters. The lowest total fungal counts (12.26×10^3 CFUs/g) were recorded in 0.85 a_w sample at the start of storage, whereas the highest total viable counts i.e. 18.01×10^3 CFUs/g were documented at day 45 in the grain sample of 0.95 a_w. The total *Fusarium* count varied from 0.42×10^3 CFUs/g in control sample at the start of storage time to 0.94×10^3 CFUs/g at day 45 in the sample modified to 0.95 a_w. The lowest ZON (855.50 µg/kg), DON (1246.45 µg/kg), 3-ac DON (234.41 µg/kg), 15-ac DON (90.58 µg/kg), T-2 (221.15 µg/kg) and HT-2 (221.24 µg/kg) were recorded at the onset of storage time in the sample of 0.85 a_w, whereas these mycotoxins steadily increased and attained the highest values at the uppermost marginal values of a_w and storage time. It was concluded that fungal growth and mycotoxins production in maize grains were favored by elevated a_w and increasing storage time. Therefore, it is recommended that cereals grains should be kept at reduced a_w to prevent fungal growth and subsequent mycotoxins production in the grains during post-harvest storage.

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Introduction

Maize (*Zea mays L.*) is the third major cereal crop after wheat and rice in Pakistan (Tanveer *et al.,* 2014). Maize grains are used in the production of edible oils, animal feed and alcohol in addition to direct human consumption. In Pakistan maize was cultivated on a total area of 1.34 million hectares in 2016-2017 with a total production of 6.134 million tonnes (MNFSR, 2017). In Pakistan, the maize is mainly grown in Khyber Pakhtunkhwa and Punjab. The crop is usually grown two times in a year during July to September and February to March.

Maize crop is infected by several genera of fungi including *Penicillium*, *Aspergillus*, *Fusarium, Rhizopus* and *Mucor* during the pre- and post-harvest stages (Hell et al., 2000; Nesci et al., 2003; Abbas et al., 2006). Maize infestation by *Fusarium* species starts from standing crop and prevails during post-harvest storage of grains (Krnjaja et al., 2013). Deleterious species of *Fusarium* such as *Fusarium langsethiae, F.*

graminearum, F. poae, F. culmorum, F. sporotrichioides, F. crookwellense and *F. equiseti* secrete toxic secondary metabolites known as mycotoxins (Trung et al., 2001). Toxicologically important *Fusarium* mycotoxins are categorized into zearalenone (ZON), trichothecenes family [i.e. trichothecenes type-A comprised of T-2 toxin (T-2) and HT-2 toxin (HT-2) and trichothecenes type-B include deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ac DON), 15-acetyldeoxynivalenol (15-ac DON)] and fumonisins (Antonissen *et al.,* 2014). Recent survey conducted by BIOMIN across 72 countries of the world showed the infection of maize by mycotoxins especially trichothecenes, zearalenone and fumonisin (Taschl and Jenkins, 2018). Temperate climatic conditions favor *Fusarium* infestation of cereal crops, since they need comparatively lower temperature for their growth and mycotoxin production than other toxigenic fungi, for instance, *Aspergillus* species.

Fusarium species attack maize crop at any stage of production, harvesting and storage. Their infestation result in substantial quantity and quality deterioration of maize grains and cause sufficient economic losses to the farming community. The major factors that influence *Fusarium* infection and their subsequent production of mycotoxins include environmental relative humidity, temperature, moisture availability or water activity (a_w) of the grains, pre- and postharvest handling, infestation and physical damage caused by insects and storage structure (Sanchis and Magan, 2004).

The most relevant parameter when considering moisture in relation to fungal growth is not the water content of a substrate but the water activity. It is a prime tool practiced for care and increasing shelf life of food and feed with respect to physicochemical properties, speed of degradative reactions and microbial growth (Vega-Mercado and Barbosa-Canovas, 1994). Water activity represents the free water content that is not physically or chemically bound to the substrate and is therefore instantly available for microbial growth. Fungal growth is therefore directly related to water activity rather than water content *per se* (Magan and Olsen, 2004). The a_w of a hygroscopic substance is equivalent to its per cent equilibrium humidity and is measured on a scale from 0.0 (completely dry) to 1.0 (pure water). Most of the food spoilage fungi cannot grow at a_w level less than 0.70, for maize this corresponds to 15% moisture content (Multon, 1988). In an *in vitro* study Hope and Magan (2003) found that the range of a_w for mycotoxins production was narrower than the range for the growth of fungi. They also demonstrated that mycotoxin production was enhanced by increasing a_w level. Likewise, there is a positive relation between mycotoxin accumulation in grains and storage length (Kaaya and Kyamuhangire, 2006; Liu et al., 2006).

In the forth-going situation of *Fusarium* mycotoxin contamination in Pakistani maize (Khatoon et al., 2012), this research was carried out to determine the impact of a_w and storage time on total fungal and *Fusarium* population and production of the mycotoxins ZON and trichothecenes type-A (T-2 and HT-2) and trichothecenes type-B (DON, 3-ac DON, 15-ac DON) in maize grains during storage.

Materials and Methods

Water activity modification and storage of maize grains

This study was conducted at the Department of Agricultural Chemistry, the University of Agriculture Peshawar during 2017-18. Maize grain composite sample (5 kg) was obtained from Agricultural Research Farm, the University of Agriculture Peshawar. The experiment was designed in completely randomized design with two factors. The factors included a_w at levels of 0.95, 0.90 and 0.85 and incubation period at 0, 15, 30 and 45 days of storage. Sterilized distilled water was used for the rehydration (i.e. a_w modification) of maize grain samples. The samples (400 g each) were taken in sterilized food jars (Ampula Ltd, UK) having porous lids and water was added to them. The required quantity of water to attain the desired a_w levels of the sample was calculated from the moisture sorption isotherm prepared for maize grains (Figure 1). Initially the jars kept at 4°C for 48 hrs with intermittent shaking for uniform absorption of water by the grains. The control treatment was kept without addition of water. A total of twelve replicate jars were prepared for each treatment a_w level and incubated at 25°C in closed containers. A beaker filled with glycerol-water solution at the same a_w level was kept alongside the sample jars in order to sustain uniform equilibrium relative humidity (ERH) inside the containers. The containers were tightly closed to prevent external contamination to maintain uniform ERH throughout the experimental period. Three replicate jars were sampled at the start (day-1) and at 15, 30 and 45 days of storage and the grains

were analyzed for total fungal and *Fusarium* count, zearalenone and trichothecenes concentration.

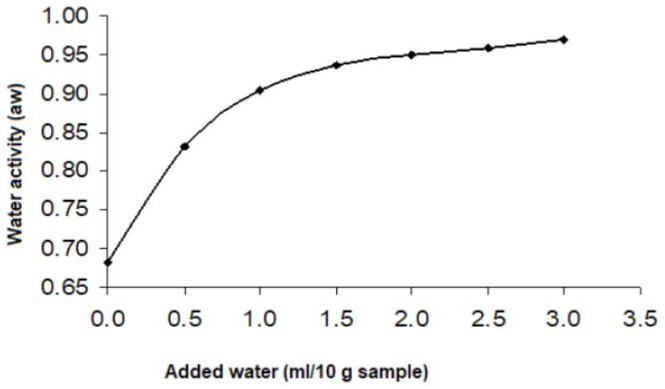

Figure 1: *Moisture sorption isotherm for maize grains (water added ml vs water activity a_w of maize).*

Total fungal and fusarium count

Total fungal count of the grain sample was determined by the method of Alam et al. (2014) using malt extract agar media. One gram well ground sample was taken in Universal glass bottle that contained 9 ml sterilized distill water and 0.01 % Tween 80. A dilution series ranged from 10^{1}-10^{-5} was made by using one in 9 ml after mixing the contents with Worley mix. An aliquot of 100 µl the sample suspension was spread on the media in Petri plates using sterilized bent Pasteur pipettes. The petri dishes were kept in the dark for one week at 25°C. After the incubation period, fungal colonies were counted and the total fungal count per gram of sample was calculated by using the following formula:

$$Total\ Fungal\ Count\ (CFUs/g) = \frac{Mean\ Colony\ count\ \times Dilution}{Volume\ of\ Liquid}$$

Similar procedure was adapted for the calculation of total *Fusarium* count of the samples where only the *Fusarium* colonies were counted excluding the colonies of other fungal species.

Determination of zearalenone and trichothecenes

The determination of mycotoxins i.e. ZON, DON, 3-ac DON, 15-ac DON, T-2 and HT-2 was carried out by modified procedures of Ostry and Skarkova, (2000) and Hanif et al. (2006) using High Performance Thin Layer Chromatography (HPTLC).

Chemicals and reagents

The standards of the above-mentioned mycotoxins were acquired from Biopure, Austria. The solvents used for HPTLC were of gradient grade and were obtained from Merck, Germany. Immunoaffinity cleanup columns ZON, DON, 3-ac DON, 15-ac DON, T-2 and HT-2 Test™ from Vicam (Watertown, MA, USA) were used for the sample clean-up.

Sample extraction and cleanup

The grains were finely grounded using laboratory grinder to pass through a 20-mesh size. Mycotoxins extraction from the sample was carried with a mixture of water and acetonitrile (16:84 v/v). About 20 g well ground sample was mixed with 50 ml of the extraction mixture and blended in high speed laboratory blender for 5 minutes. The suspension was then passed through Whatman filter paper to separate the filtrate. Aliquots of the filtrates (2 ml each) were loaded on immunoaffinity cleanup columns designated for each mycotoxin. The columns were washed with purified water (5 mL) and then vacuum dried for 3 minutes. The target mycotoxins were eluted with methanol (1 ml) into a 1.8 ml vial.

High performance thin layer chromatography

An aliquot of 20µl of cleaned sample extract was applied to silica gel thin layer chromatographic plates along with diluted calibration standards of mycotoxins. The TLC plates were eluted and developed with a mixture of propanol-acetone-chloroform (1:1:8 v/v) in dark in a 10 x 20 cm well saturated horizontal chamber by HPTLC. The plated were dried with gentle blow of cold air and then sprayed with solution of aluminum chloride in ethanol (20 %) and heated at 105°C for 10 min. After developing the plates, a fluorescence densitometry with a Camag TLC Scanner II having mercury lamp and K 400 secondary filter was used to determine the concentration of mycotoxins. The excitation and emission wavelengths were 366 and 420 nm, respectively.

Statistical analysis

The data were statistically analysed using two factor Completely Randomized Design (CRD) by statistical software Statistix 8.1. The significant differences among treatment means were calculated by Least Significant Difference (LSD) test for the main as well as interaction effect (Steel et al., 1996).

Results and Discussion

Total fungal count of maize grains

The data presented in Figure 2 show the total fungal

count of maize grains samples at different a_w levels and at storage duration of 0, 15, 30 and 45 days. It was observed that a significant ($p<0.05$) increase in total fungal count was occurred with increase in water activity and storage time. The lowest total fungal counts (12.26×10^3 CFUs/g) were recorded in control sample at the start of storage time (i.e. day-1), whereas the highest total viable fungal count i.e. 18.01×10^3 CFUs/g were documented in the sample modified to $0.95a_w$ at day 45 of storage.

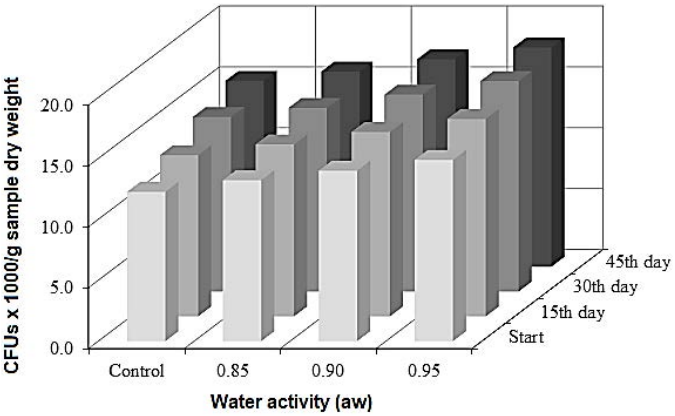

Figure 2: *Total fungal count (CFUs*1000/g) of maize samples at different water activity (a_w) levels (control, 0.85, 0.90 and 095a_w) at different storage intervals (start, 15, 30 and 45 days).*

Total fusarium count of grains

The data regarding the total *Fusarium* count of maize grains samples stored at a_w levels of 0.85, 0.90 and 0.95 a_w for 45 days are shown in Figure 3. It was observed that significant increase ($p<0.05$) occurred in the total *Fusarium* count with increase in water activity and storage time. The total *Fusarium* count ranged from 4.20×10^2 CFUs/g to 5.40×10^2 CFUs/g in control and 0.95 a_w samples, respectively. The data concerning storage time showed that total *Fusarium* count ranged from 4.20×10^2 CFUs/g to 9.40×10^2 CFUs/g; the lowest in control sample at the start of storage time while the highest in 0.95 a_w sample at day 45.

Zearalenone content of maize grains

Table 1 represent the data regarding the ZON contents of maize grain samples at storage interval of 0, 15, 30 and 45 days in response to water activity modification at 0.85, 0.90 and 0.95 a_w levels. Statistical analysis of the data showed that both a_w and storage duration had significant influence on ZON production in maize grains. The highest content of ZON (928.55 µg/kg) was recorded at 0.95 a_w at 45 days interval, whereas the lowest concentration (855.50 µg/kg) was analyzed in the control sample at the start of storage. An increasing trend of ZON contents was observed with respect to increasing a_w and storage time whereas the interactive impact of a_w and incubation time was non-significant ($p>0.05$).

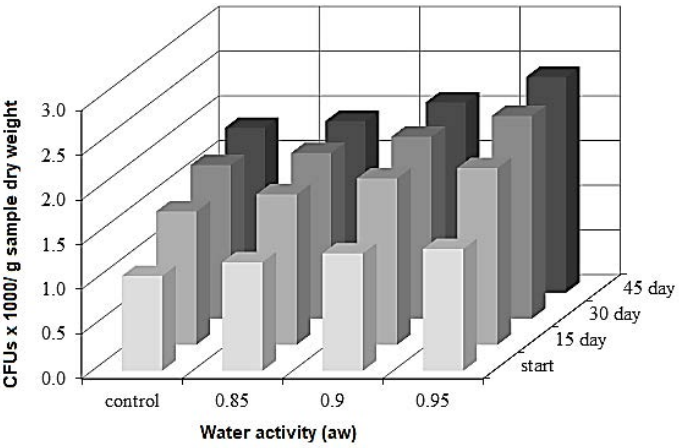

Figure 3: *Total fusarium count (CFUs*1000/g) in maize samples at different water activity (a_w) levels (control, 0.85, 0.90 and 095) at different storage time (start, 15, 30 and 45 days).*

Trichothecenes mycotoxins

There are two types of trichothecenes mycotoxins i.e. type-A and type-B. The type-A trichothecenes includes HT-2 and T-2 whereas the type-B consists of DON, 3-ac DON and 15-ac DON.

HT-2 content

Table 2 presents the HT-2 contents of maize grain samples stored at 0.85, 0.90 and $0.95a_w$ for 45 days. The data showed that the HT-2 contents of maize samples significantly ($p<0.05$) varied with a_w levels and storage time. The control sample exhibited the lowest HT-2 contents (221.24 µg/kg) at day-1 of storage time while the highest content (303.80 µg/kg) was recorded at 0.95 a_w after 45 days of storage. The data regarding the effect of storage time showed that an increase in HT-2 content occurred from an average value of 244.98 at day-1 to 277.17 µg/kg at day 45 of storage. Likewise, the HT-2 content of the grains steadily increased with increase in water activity.

T-2 content

Table 3 represents the data pertaining to T-2 mycotoxin content of maize samples in response to a_w modification and storage for a period of 45 days. The data concerning T-2 contents in maize grains varied significantly ($p<0.05$) with water activity alteration and storage time. The lowest T-2 contents (221.15 µg/kg) was recorded in control sample at the onset of storage time which progressively increased to 251.13 µg/kg

Table 1: *Zearalenone contents (µg/kg) of maize grains at water activity level of 0.85, 0.90, 0.95a_w at 1, 15, 30 and 45 days of storage.*

Water activity (a_w)	Time Interval (days)				Mean
	1	15	30	45	
Control	855.50±1.50	875.47±2.39	892.29±2.30	899.28±3.40	880.64 ±4.20d
0.85	875.21±2.50	887.59±2.08	903.22±2.10	908.84±2.90	893.72±4.04c
0.90	891.18±2.25	896.94±2.25	909.78±2.15	920.48±3.12	904.60±3.95b
0.95	905.71±2.75	909.22±2.30	923.19±2.34	928.55±3.42	916.67±4.12a
Mean	881.90±2.75d	892.31±2.40c	907.12±2.38b	914.29±3.34a	

Means in each row and column followed by different letters are significantly different at p≤0.05.

Table 2: *HT-2 contents (µg/kg) of maize grains at water activity level of 0.85, 0.90, 0.95a_w at 1, 15, 30 and 45 days of storage.*

Water activity (a_w)	Time Interval (days)				Mean
	1	15	30	45	
Control	221.24±1.45	231.23±1.88	241.16±1.98	251.14±2.04	236.19±2.33d
0.85	236.25±1.85	247.17±2.05	258.13±1.94	268.04±2.12	252.40±2.35c
0.90	251.29±1.75	262.58±1.95	275.20±2.23	285.71±1.99	268.69±2.23b
0.95	271.16±1.54	282.40±2.06	293.81±2.12	303.80±2.11	287.79±2.14a
Mean	244.98±2.34d	255.85±2.43c	267.07±2.45b	277.17±2.55a	

Means in each row and column followed by different letters are significantly different at p≤0.05.

Table 3: *T-2 contents (µg/kg) of maize grains at water activity level of 0.85, 0.90, 0.95a_w at 1, 15, 30 and 45 days of storage.*

Water activity (a_w)	Time Interval (days)				Mean
	1	15	30	45	
Control	221.15±1.54	230.95±1.66	241.15±1.78	251.13±1.68	236.10±1.68d
0.85	231.00±1.74	239.02±1.58	249.83±1.89	259.12±1.79	244.74±1.87c
0.90	236.78±1.86	245.76±1.65	254.65±1.95	263.71±1.93	250.23±1.96b
0.95	240.96±1.95	251.14±1.96	260.92±2.06	271.03±1.87	256.01±1.99a
Mean	232.47±1.98d	241.72±2.14c	251.64±2.21b	261.25±2.18a	

Means in each row and column followed by different letters are significantly different at p≤0.05.

after 45 days of storage. Similarly, the samples which were modified to 0.95a_w showed 240.96 µg/kg T-2 mycotoxin that steadily increased to 271.03 µg/kg after 45 days of storage.

Deoxynivalenol content

The data related to the DON mycotoxin content of maize grains samples stored at 0.85, 0.90 and 0.95 a_w levels for 45 days are shown in Table 4. Analysis of variance indicated that both a_w and storage duration alone and in combination significantly ($p<0.05$) affected DON production maize grains. Generally, an increasing trend of the mycotoxin production was noted with increasing level of a_w and storage time. The lowest mean value (1246.45 µg/kg) was recorded in control sample, while the highest (1377.47 µg/kg) was documented at 0.95a_w. The data concerning the storage time indicated that lowest mean value of DON contents (1230.02 µg/kg) in maize samples was recorded at day-1 followed by 15 days (1281 µg/kg), 30 days (1358.7 µg/kg) and the highest (1397.51 µg/kg) was recorded at 45 days of storage.

3-ac DON content

The data regarding 3-ac DON in maize samples at different water activity levels and different storage time showed increasing trend as the water activity and storage time increased (Table 5). Significant ($p<0.05$) variation was observed in 3-ac DON contents at different water activity levels. The maximum value (271.71 µg/kg) was recorded at 0.95 a_w and the minimum (234.41 µg/kg) was noted in control sample.

Table 4: *DON contents (µg/kg) of maize grains at water activity level of 0.85, 0.90, 0.95a$_w$ at 1, 15, 30 and 45 days of storage.*

Water activity (a_w)	Time Interval (days)				Mean
	1	15	30	45	
Control	1151.48±2.98	1211.48±3.18	1291.50±3.45	1331.33±3.46	1246.45±3.23d
0.85	1211.14±2.15	1260.95±3.65	1341.10±3.65	1381.25±3.73	1298.61±3.45c
0.90	1261.18±3.18	1306.05±3.22	1386.18±2.98	1426.21±3.98	1344.91±3.65b
0.95	1296.28±3.25	1345.99±3.24	1416.34±3.55	1451.25±4.04	1377.47±3.98a
Mean	1230.02±4.86d	1281.12±4.67c	1358.78±4.78b	1397.51±4.54a	

Means in each row and column followed by different letters are significantly different at p≤0.05.

Table 5: *3-ac DON contents (µg/kg) of maize grains at water activity level of 0.85, 0.90, 0.95a$_w$ at 1, 15, 30 and 45 days of storage.*

Water activity (a_w)	Time Interval (days)				Mean
	1	15	30	45	
Control	211.25±1.56	231.26±1.76	241.95±1.87	253.16±1.79	234.41±185d
0.85	233.04±1.65	243.26±1.89	254.28±1.96	264.98±1.98	248.89±1.97c
0.90	244.22±1.88	255.32±1.99	266.43±1.78	276.98±2.03	260.74±2.08b
0.95	257.26±1.99	267.16±2.11	276.45±1.94	285.98±2.32	271.71±2.43a
Mean	236.44±2.14d	249.25±2.35c	259.78±2.09b	270.28±2.56a	

Means in each row and column followed by different letters are significantly different at p≤0.05.

Table 6: *5-ac DON contents (µg kg^{-1}) of maize grains at water activity level of 0.85, 0.90, 0.95a$_w$ at 1, 15, 30 and 45 days of storage.*

Water activity (a_w)	Time Interval (days)				Mean
	1	15	30	45	
Control	80.98±1.44	87.24±1.58	93.98±1.68	100.11±1.65	90.58±1.78d
0.85	87.55±1.65	93.88±1.66	100.65±1.58	107.92±1.76	97.50±1.94c
0.90	94.84±1.78	101.64±1.87	109.03±1.76	114.92±1.58	105.11±1.76b
0.95	100.85±1.89	107.91±1.98	114.72±1.67	120.88±1.87	111.09±2.12a
Mean	91.05±2.01d	97.67±1.99c	104.60±2.23b	110.96±1.96a	

Means in each row and column followed by different letters are significantly different at p≤0.05.

The data concerning the 3-ac DON contents with respect to time storage showed highest (270.28 µg/kg) value at days 45 and lowest (236.44 µg/kg) was recorded at the start of storage.

15-ac DON content

Table 6 represents the data regarding 15-ac DON in maize grain samples at different water activity levels and at different time storage. The data recorded for water activity showed a range of average values from 90.58 µg/kg to 111.09 µg/kg. The minimum value was recorded in control sample and the maximum was noted at 0.95 a$_w$. Similarly, the mean highest value (110.96 µg/kg) for time storage was recorded at 45 day of storage and lowest (91.05 µg/kg) was noted for 15-ac DON contents in maize samples at the start of storage time.

To study the effect of water activity and storage time, the samples of maize were modified to various water activity levels (0.85, 0.90 and 0.95 a$_w$) and stored for 45 days under ambient room temperature of 25°C. The samples were analyzed for total fungal count, total *Fusarium* count and mycotoxins ZON, HT-2, T-2, DON, 3-ac DON, and 15-ac DON at different storage intervals i.e. 0, 15, 30 and 45 days of storage.

The results regarding total fungal counts in maize grain samples showed that the lowest total viable fungal counts i.e. 12.26×10^3 were recorded at the start of experiment (day-1 of storage) in control (a$_w$ not modified) samples. The total fungal count increased with increase in a$_w$ and storage duration. The highest values for total fungal population was recorded for

the samples of maize at incubation period of 45-day and water activity level 0.95a_w. The present results are fairly in line with findings of Marin et al. (2000) and Suhr and Nielsen, (2004). They demonstrated that sufficient availability of water enhanced the total fungal population of stored maize grains. In other studies, conducted by Beg et al. (2006), Zinedine et al. (2007) on broiler feed having corn grains as vital ingredient reported that fungal colonization and ultimately mycotoxins accumulation was a serious issue that was affected by various factors including water availability and length of storage time. Fungal infection in cereal grains is one of the serious issues that are responsible for the reduction of quality and quantity of grains as well as cause huge economic losses to human societies. Fungal infestation of food and feed also results in the production of a number of mycotoxins and threatens humans and animals' health (Manizan et al., 2018). Reasonably warm and humid climatic environment, poor post-harvest management and insufficient storage practices facilitate the fungal growth and mycotoxins production (Hell et al., 2000; Klich, 2007). There are several methods which can be used for the control of fungal growth in cereals e.g. thermal inactivation, irradiation, enzymatic and microbial degradation, use of chemical preservatives and reducing the water activity of the substrate (Byun and Yoon, 2003; Haque et al., 2009; Alam et al., 2014).

The results of our study exhibited that as the water activity of the grains increased from control to 0.95 a_w, the total *Fusarium* count also increased. The highest total *Fusarium* count i.e. 9.40×10^3 CFUs/g was recorded at 0.95 a_w after 45 days of storage. Our results are in line with those of Jayas and Jeyamkondan, (2002) and Mannaa and Kim (2017) who studied the influence of a_w and temperature on fungi and their mycotoxins secretions in stored grains. They reported that *Fusarium, Alternaria* and *Epicaccum* species are hygroscopic in nature and increase their mycelial growth at high water activity level i.e. 1.00 a_w. Marin and Magan (1999), Sanchis and Magan (2004) and Waskiewicz et al. (2010) also reported that *F. graminearum* and *Fusarium spp* grow best at water activity level of 0.98 a_w and produce maximum zearalenone, fumonisins and deoxynivalenol contents in cereal grains at higher a_w levels and at temperature range of 18-25°C. *Fusarium* species are amongst the most common plant pathogens which attack the crops at various stages of crop cycle. Their preferred hosts include cereals like wheat, maize, rice, oat, barley and triticale. They infect different parts of the plant including seedlings, heads, roots and stems and cause a variety of diseases e.g. Fusarium head blight, Fusarium wilt and Fusarium root rot (Balmas et al., 2015). These diseases are the main cause of economic losses which are estimated in billions of dollars worldwide. In a study conducted in Europe the main diseases caused in cereal crops were due to the infection of different *Fusarium* species like *F. graminearum, F. crookwellence, F.poae, F. sporotriochiodes, F. langsethiae, F. seudograminearum,* and *F. equiseti* (Botallico, 2002; Vogelgsang et al., 2008). There are several reports which describe the infection of *Fusarium* species on winter corn with relation to climatic conditions and crop alternation (Xu et al., 2013; Czembor et al., 2015).

Fungal infestation also deteriorates the crop quality by producing mycotoxins which results in the degradation of the quality and quantity of cereal grains (Champeil et al., 2004; Wegulo et al., 2015). The *Fusarium* mycotoxins contamination in cereals is a major problem for food safety and security throughout the world. Our results showed that the samples of maize had an increased level of ZON from 35.32 µg/kg in control sample to 928 µg/kg in the sample at 0.95a_w at 45-day of storage. Similarly, the results for DON had the highest value recorded in maize samples which was maximum (1397 µg/kg) at 45-day and 0.95 a_w. The results of Milani (2013) and Neme and Mohammad (2017) are in close agreement to our findings. They also noted increase in ZON and DON contents with increase in a_w levels. Likewise, increasing pattern was noted for HT-2, T-2, 3-ac DON and 15-ac DON with increase in water activity and incubation time in all the samples of maize. Sanchis and Magan (2004) and Alam et al. (2009) reported that the optimum temperature for growth and aflatoxin production by *Aspergillus* range from 25 to 30°C and best water activity level is 0.95 a_w.

Conclusions and Recommendations

It was concluded that lower water activity level and shorter storage time restricted total fungal and *Fusarium* population, while increased level of water activity (0.95 a_w) and longer storage time (45-days) provided conducive environment for total fungal and *Fusarium* growth which inevitably increased the production of mycotoxins zearalenone and trichothecenes in maize grains.

Novelty Statement

This is the first study of its type to investigate the combined effect of water activity and storage time on Fusaria population and their associated mycotoxins production in maize grains in Pakistan.

Author's Contribution

Fazle Akbar conducted the experimental work, collected and analyzed the data and wrote the manuscript. Sahib Alam designed and supervised the study, provided overall inputs and conceived the idea of the research.

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