DETERMINATION AND DETOXIFICATION OF TOTAL AFLATOXINS AND DEOXYNIVALENOL FROM DIFFERENT TYPES OF CEREALS AND PULSES COLLECTED FROM DIFFERENT AREAS

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Abstract. Mycotoxin contamination of food products poses a major risk to both human and animal health. In addition to posing a threat to human health and food safety, mycotoxins have a significant detrimental economic impact because of their effects on international trade, crop yields, and animal production. To ensure safe food, it is therefore vital to identify, measure, and reduce mycotoxins in food commodities, especially aflatoxins and deoxynivalenol (DON) in cereals and pulses. The current research investigated the concentrations of total aflatoxins and DON in a total 45 samples of cereals and pulses collected from different markets of Lahore, Pakistan, and assessed the utility of different physical and natural methods to minimize their levels. Enzyme-linked Immunosorbent Assay (ELISA) technique was verified to identify aflatoxins and DON. ELISA is the easiest sensitive method for the quantification of mycotoxins in food entities. The results showed that 10 of 45 samples (22%) exceeded the EU regulatory limit for total aflatoxins, while 32 samples (71%) exceeded the EU (2023) regulatory limit for DON contaminants. The variety with the maximum concentration of total aflatoxins was wheat grains, while brown chickpeas had the maximum DON concentration. Also, there was a significant difference between total aflatoxins and DON in pulses samples at $p < 0.05$. On the other hand, physical detoxification treatments reduce such toxins by 25–46%, whereas natural detoxification treatments reduce them by 34–64% in which lemon juice showed effective removal of both total aflatoxins and DON contaminants. Thus, it is concluded that contamination of cereals and pulses with total aflatoxins and DON was quite high in the samples collected in Lahore, Pakistan. Furthermore, natural detoxification methods can greatly decrease the contamination of mycotoxins in cereals and pulses.
Keywords: *mycotoxins, aflatoxins, deoxynivalenol, pulses, cereals, elisa*

Introduction

Pakistan is among the world's leading producers and suppliers of food and agricultural products. Pakistan's economy is largely dependent on its principal crops, and agriculture is regarded as its backbone (Rehman et al., 2015). Major pulse crops, such as chickpea (Cicer arietinum L.), lentil (Lens culinaris Medic.), mung bean (Vigna radiata (L.) Wilczek), black gram or mash bean (Vigna mungo L. Hepper), are grown on around 1.3 million hectares of land in Pakistan. Chickpeas are the main winter food

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legume among these pulses, while mung is the main summer legume (Yasmeen et al., 2017). The majority of people on the planet eat cereals like corn, wheat, and barley as their main source of nourishment and energy. Pakistan is a tropical country, thus the climate there promotes the growth and production of fungi (Majeed et al., 2017). Mold contamination of crops, particularly cereals, during the pre-harvest and post-harvest phases can result in the generation of mycotoxins, which are secondary hazardous metabolites (Silva et al., 2022; Lima et al., 2022). The primary producers of mycotoxins are Aspergillus, Penicillium, Alternaria, Claviceps, Fusarium species, and aflatoxins, deoxynivalenol, trichothecenes, fumonisins, and ochratoxin A (Udovicki et al., 2018).

Among these are fungal compounds called aflatoxins, which are produced by fungus species such as Aspergillus flavus, Aspergillus nomius, and Aspergillus parasiticus (Smith et al., 2020; Sadaf et al., 2023). These are most frequently associated with a range of diverse agricultural food products as well as different cereals such as corn, rice, wheat, groundnuts, dried fruits, cocoa beans, and vegetable oils (Khodaei et al., 2021; Sarma et al., 2017). The main forms of aflatoxins that can infect food are B1, B2, G1, and G2 (Dors et al., 2011). Aflatoxin toxicity levels are ranked as follows: AFTB1 > AFTG1 > AFTB2 > AFTG2 (Kumar et al., 2017). The most common aflatoxin is aflatoxin B1, which is classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC) (Azizi et al., 2013). When aflatoxins are detected in excess of allowable limits, they can result in serious, acute, and chronic illnesses in both people and animals (Marin et al., 2013). Mycotoxins not only endanger human health and food safety, but they also have a major negative economic impact due to their effects on crop yields, animal productivity, and international trade (Alshannaq and Yu, 2017). The maximum amounts of aflatoxins that are allowed vary by nation (Cucci et al., 2007). Aflatoxin levels in processed foods are allowed to be 10 μg/kg in more than 75 countries (Herzallah et al., 2009). Nonetheless, the European Union (EU) permits aflatoxins at levels between 2 and 4 μg/Kg.

Deoxynivalenol (DON), also known as vomitoxin (type B trichothecene), is another form of mycotoxin that is frequently found in cereals like corn, wheat, and barley (Machado et al., 2017; Medina et al., 2019). Fusarium species, including F. graminearum and F. culmorum, are the primary producers of DON. When DON was initially isolated in 1972 from moldy barley in Japan, it was discovered to be the same as the emetic component discovered in corn in the United States (Richard et al., 1993). It has been estimated that mycotoxins contaminate between 25 and 50 percent of the harvested crops worldwide each year (Ricciardi et al., 2013). For both raw and processed grain products, various nations and organizations have set maximum DON levels. A permitted limit of 2000 μg/kg has been determined for uncooked barley, wheat, and corn by the Codex Alimentarius Commission. In a similar vein, unprocessed wheat and oats have a 2000 μg/kg limit set by the EU. However, for raw or unprocessed grains, there is an allowed regulatory limit of 1000 μg/kg (European Commission, 2006). A daily dietary limit of 1 μg/kg bw is suggested for DON.

Several types of management interventions, including appropriate agricultural practices, optimal storage conditions, and the setting of legislative restrictions in cereal grains and feed, have been explored in an effort to reduce mycotoxin formation and prevent the negative effects that follow (Streit et al., 2012). Various chemical and physical methods have been used globally to lower mycotoxin levels in food commodities (Sarrocco et al., 2018; Tsitsigiannis et al., 2012). Nevertheless, the decontamination efficacy of chemically treating food items to remove mycotoxin is

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poor (Maxwell et al., 2006). Therefore, the development of some workable and efficient decontamination approaches is required. Accordingly, it is believed that the best postharvest way for lowering mycotoxins in food during storage is the use of physical measures (Karlovsky et al., 2016).

There is not much information on the toxicological data of mycotoxins in crops accessible in Pakistan. Since the significance of mycotoxin presence in Pakistan's various crops, an evaluation of the mycotoxin contamination level is required. Thus, the goal of the current study is to optimize physical and natural detoxification techniques for the highly contaminated samples while also updating data on aflatoxins and DON levels in Pakistani cereals and pulses. Farmers, merchants, and other stakeholders in Pakistan will benefit from this information being spread through the evaluation of total aflatoxins and DON levels in grain and pulse products.

Materials and methods

The cereals samples of two types including ten samples of wheat grains and ten samples of corn; and pulses of five types including five samples of red lentils, five samples of brown lentils, five samples of yellow lentils, five samples of black eye peas and five samples of brown chickpeas were randomly collected from local markets of Lahore, Pakistan in order to determine the contamination of aflatoxins and deoxynivalenol in these samples. Lahore is one of Pakistan's main centers of industry, education, and the economy. It is one of the most progressive, cosmopolitan, and socially liberal cities in Pakistan and has served as the historical capital and cultural hub of the larger Punjab province. It is also called as the city of food. So, Lahore was selected for the study of mycotoxins contamination in different cereals and pulses. Total 45 samples of cereals and pulses were analyzed by using Enzyme-linked Immunosorbent Assay (ELISA) for the determination of total aflatoxins and DON.

Extraction of aflatoxins

Aflatoxin levels in samples were evaluated using the Veratox ELISA Quantitative Kit (Product 8030, Neogen, USA). The samples were entirely crushed after mixing. The samples were kept at 2-8°C (35-46°F) until analysis. The AOAC Method was employed to extract aflatoxins. 50 grams of ground sample was placed in 250 mL of 70% methanol and swiftly shaken for 3 min. 5 mL of extract was filtered.

Extraction of DON

DON levels in samples were evaluated using the Veratox ELISA Quantitative Kit (Product 8335, Neogen, USA). The samples were entirely crushed after mixing. The samples were kept at 2-8°C (35-46°F) until analysis. 50 ± 0.2 g of sample was taken and added 250 mL of distilled water in conical flask. The sample solution was swiftly shaken for 15 min. 5 mL of extract was filtered.

ELISA testing for aflatoxin

The chemicals used in the experiment were let to settle at room temperature (18-30°C or 64-86°F). Four red-marked standard mixing wells and one red-marked sample mixing well per sample were arranged in a well holder for testing purposes. Similarly, identical numbers of antibody-coated wells were added on a micro-well plate. Prior to usage, all of the

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reagents in the reagent bottles were thoroughly mixed. To every red-marked mixing well, 100 μL of conjugate from the bottle with the blue label was added. All samples and controls were also transferred in triplicate, in 100 μL, to the red-marked mixing wells. The substance in the red-marked wells was thoroughly stirred by pipetting it up and down several times. 10 0μL solution was transferred from the red mixing wells to the antibody-coated wells. To mix the wells, the antibody-coated wells in the holders/plate were shifted back and forth for 2 min. The contents of the antibody-wells have been dumped out. To ensure that the water was thoroughly cleaned, distilled water was poured into each well and then emptied out at least five times. On paper or a towel, each well was tapped out to remove any remaining water. The wells were filled with 100 μL of substrate and vigorously shaken for 3 min. A 100 μL quantity of red stop solution was added, and it was thoroughly mixed by moving the wells-plate back and forth. A 650 nm filter was employed in the microwell reader to read the plate. The data analysis of total aflatoxins concentration was performed using Neogen's Veratox software and Stat Fax microwell reader.

ELISA testing for DON

Five red-marked standard mixing wells and one red-marked sample mixing well per sample were arranged in a well holder for testing. Then same procedure was followed as described above for aflatoxins testing.

Detoxification by physical methods

The highly contaminated cereals and pulses samples were detoxified by washing, heat in excess water and cooking treatments to lower the level of total aflatoxins and DON. These treatments were applied on three replicates for each sample.

Washing

A beaker containing 50 grams of contaminated sample was filled with 250 ml of distilled water, and the mixture was vigorously shaken for 30 min. This process was repeated three times. After that, the sample was dried and examined for total aflatoxins and DON reduction analysis.

Heat in excess water

A 500 ml beaker of water containing 50 grams of contaminated sample was set on a hotplate. For 15 min, the sample solution was heated. After filtering the solution, the sample was allowed to air dry at room temperature and tested for total aflatoxins and DON reduction analysis.

Cooking

A 500 ml beaker of water containing 50 grams of contaminated sample was set on a hotplate. At 100°C, the solution was cooked for 30 min. After filtering the solution, the sample was allowed to air dry at room temperature. The sample was tested for total aflatoxins and DON reduction analysis.

Detoxification by natural methods

The highly contaminated cereals and pulses samples were detoxified by using black seed oil, mustard oil and lemon juice to lower the level of total aflatoxins and DON.

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These natural detoxifiers were selected due to their harmlessness mentioned in different literature/research work. These treatments were applied on three replicates for each sample.

Treatment with black seed oil

Black seed oil was purchased from Gulberg market in Lahore. Black seed oil was utilized for decontaminating the sample. 90 ml of distilled water were used to dilute ten ml of black seed oil, and 50 grams of contaminated sample was put in to the mixture. The sample was immersed in a solution of black seed oil in a wooden hood at 25°C for 2 h. After filtering, the mixture was examined for the presence of DON and total aflatoxins.

Treatment with mustard oil

Mustard oil was also purchased from Gulberg market in Lahore. 90 ml of distilled water were used to dilute ten ml of mustard oil, and 50 grams of contaminated sample was put in to the mixture. The sample was immersed in a solution of mustard oil in a wooden hood at 25°C for 2 h. After filtering, the mixture was examined for the presence of DON and total aflatoxins.

Treatment with lemon juice

Fresh Lemons were bought from China Scheme market in Lahore. To get rid of all the dust and contaminants, lemons were properly cleaned with water. After washing and cutting the lemons into small pieces, the juice was squeezed into a 100 ml flask. 10 ml of lemon juice were diluted with 70 ml of distilled water. 50 grams of contaminated sample was added into the lemon juice mixture and incubated in a wooden hood at 25°C for 30 min. The solution was then filtered and analyzed for the presence of total aflatoxins and DON.

Statistical analysis

Descriptive statistics including means and standard deviation were calculated by Microsoft Excel 2016 (Microsoft Office). Paired t-test was applied separately to find the significant difference of both toxin (total aflatoxins and DON) within cereals and pulses. One-way ANOVA was applied to test the significance of different physical and natural methods on lowering both toxins in the samples.

Results

The study investigated the levels of total aflatoxins and Deoxynivalenol (DON) in the cereals and pulses samples, providing ranges to indicate the variability of contamination levels within each group. For total aflatoxins in cereals, the recorded levels ranged from a low of 0.66 parts per billion (ppb) to a high of 8.51 ppb. In pulses, the range was wider, spanning from 0.05 ppb to 6.74 ppb (*Table 3*). The bar graph showed that total aflatoxins and DON are not statistically different in cereals samples at $p > 0.05$ (*Fig. 1*). Similarly, the levels of DON were examined, revealing a range of 0.07 to 20.13 parts per million (ppm) in cereals samples and 0.08 to 22.52 ppm in pulses samples (*Table 4*). Also, the bar graph showed that total aflatoxins and DON concentrations in pulses samples are statistically different at $p < 0.05$ (*Fig. 2*). Overall

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results showed that wheat grains (32.06 ± 2.853) showed highest level of total aflatoxins and brown chickpeas (67.28 ± 1.044) showed highest level of DON contamination.

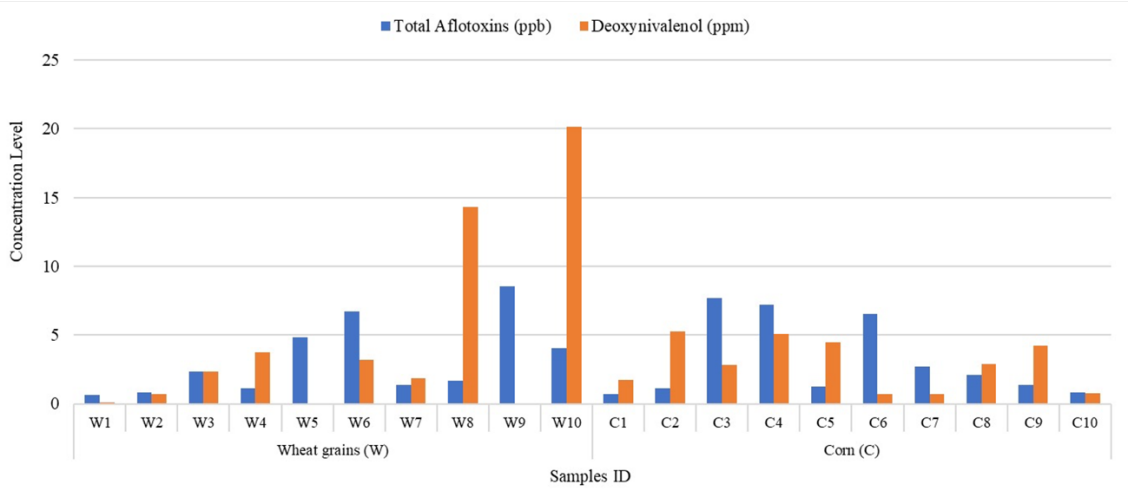

Figure 1. *Comparison of level of total aflatoxins and DON detection in cereals samples*

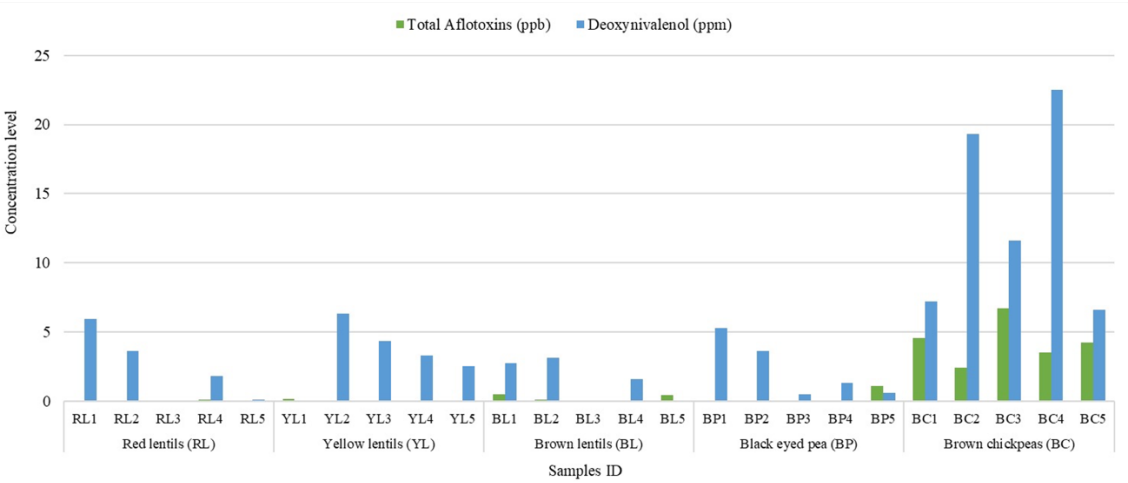

Figure 2. *Comparison of level of total aflatoxins and DON detection in pulses samples*

In case of cereals, all 20 samples tested were found to be contaminated by total aflatoxins. Of these, 7 samples (35%) exceeded the regulatory limits set by the European Union (EU). For pulses, out of the 25 samples tested, 16 samples (64%) showed contamination by total aflatoxins (*Table 1*).

Table 1. *Detail analysis of samples for total aflatoxin contamination*

Samples type	No. of samples	Contaminated samples beyond EU limit (%)	Contaminated samples within EU limit (%)	Non-contaminated samples (%)
Cereals	20	7 (35)	13 (65)	0
Pulses	25	3 (12)	13 (52)	9 (36)
Total	45	10 (22)	26 (57)	9 (20)

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For DON, 18 samples (90%) of cereals were contaminated, with 13 samples (65%) exceeding the EU limit. For pulses, 23 samples (92%) were contaminated, of which 19 samples (76%) exceeded the EU limit (*Table 2*).

Table 2. *Detail analysis of samples for deoxynivalenol contamination*

Samples type	No. of samples	Contaminated samples beyond EU limit (%)	Contaminated samples within EU limit (%)	Non-contaminated samples (%)
Cereals	20	13 (65)	5 (25)	2 (10)
Pulses	25	19 (76)	4 (16)	2 (8)
Total	45	32 (71)	9 (20)	4 (9)

Additionally, total aflatoxins contamination in wheat grain sample (W9) and DON contamination in brown chickpeas sample (BC4) was effectively reduced by the physical and natural detoxification processes. Physical methods revealed a 24.7% reduction for total aflatoxins and 27.3% for DON in washing treatment, 36.2% reduction for total aflatoxins and 31.3% for DON by heating in excess water and 46.5% for total aflatoxins and 44.4% for DON in cooking treatment (*Fig. 3*). When using natural approaches, the reduction in contaminated samples was 52.3% for total aflatoxins and 45.1% for DON when blackseed oil was used, 63.7% for total aflatoxins and 57.6% for DON when lemon juice was used and 39.4% for total aflatoxins and 34.4% for DON when mustard oil was used. In contrast to all treatments, lemon juice showed effective removal of both total aflatoxins and DON contaminants (*Fig. 4*). There is no significant difference between different physical and natural methods for the removal of both toxins at $p > 0.05$.

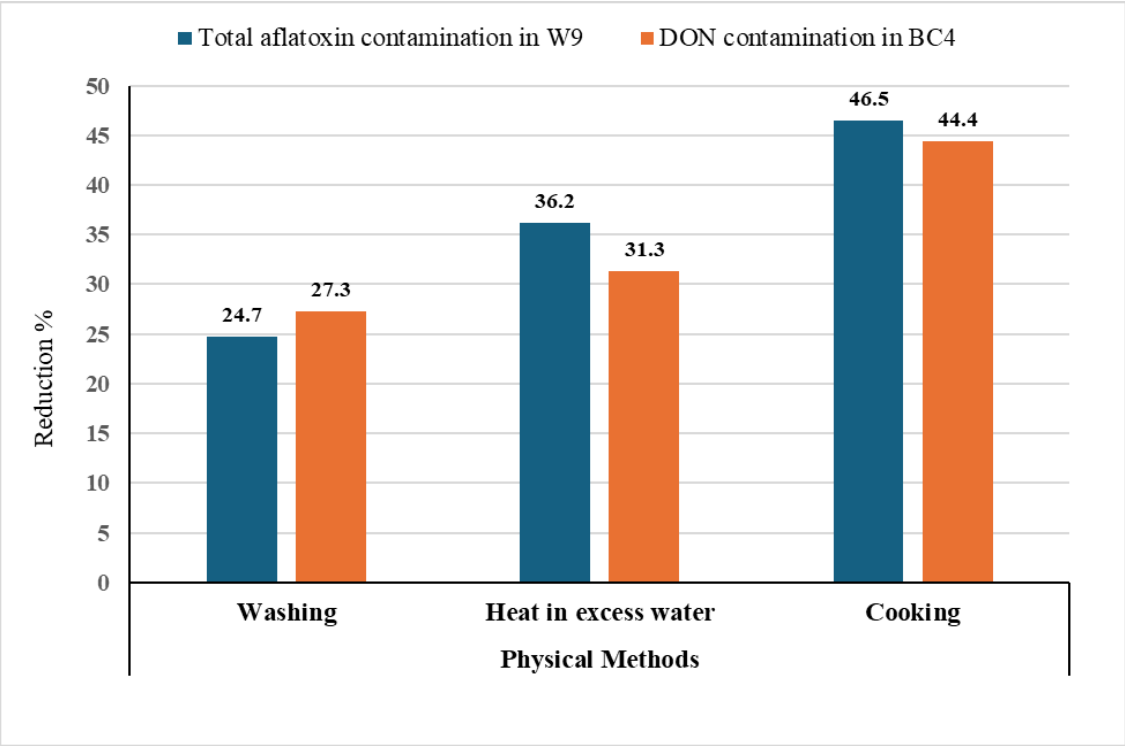

Figure 3. *Physical methods for detoxifying contaminated samples of total aflatoxins and DON*

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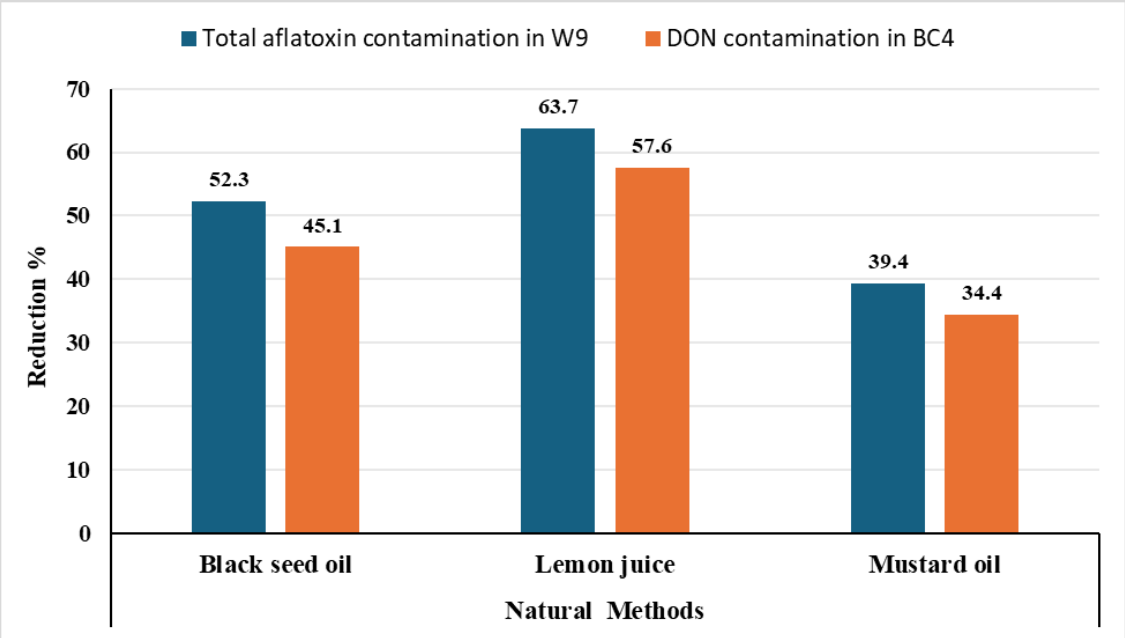

Figure 4. *Natural methods for detoxifying contaminated samples of total aflatoxin and DON*

Discussion

Cereals and pulses are valuable nutrients containing food that consumed by almost all humans and animals worldwide. Fungal attack is triggered by the rain, humidity, insufficient storage facilities, and unsuitable market environment. This nutritious food is affected by a fungal infection that produces aflatoxins and DON (Alshannaq et al., 2017). The Food and Agriculture Organization (FAO) estimates that mycotoxins induce harm to a quarter of all crops worldwide (Pankaj et al., 2018). In Pakistan due to hot and humid environment, the chances of occurrence of aflatoxins and DON are more likely in the food stock. So, being an agricultural country, there is also need to update the information about aflatoxins and DON on regular basis. In the current study, concentrations of aflatoxins and DON were tested in cereals and pulses samples of different types collected from local markets of Lahore, Pakistan. Moreover, physical and natural treatments were used to degrade these mycotoxins in highly contaminated samples.

It was found that 100% cereals samples and 64% pulses samples were contaminated with total aflatoxins. Within the positive samples, 35% cereals and 12% pulses had total aflatoxins above the EU (2023) permissible limit. The concentration of total aflatoxins in several types of cereals and pulses ranged 0.66-8.51 ppb and 0.05-6.74 ppb, respectively (*Table 3*). In Pakistan, Sadaf et al. (2023) reported that aflatoxins contamination was observed in 10%, 8.3% and 13.3% contamination in biscuit, cakes and noodles respectively. Aflatoxins were also detected highest (3.33 ppb) in the jute package with 85% moisture content as compared to HDPE (0.9 ppb) for red chilies (Abrar et al., 2023). In a study, aflatoxin contamination of 83% in branded and 91% in non-branded was noted in chocolate samples used in making cakes (Naz et al., 2017). In a report conducted in Pakistan by Lutfullah et al. (2012), the aflatoxins levels in corn, rice, wheat, sorghum and barley were determined and found the highest value i.e. 15.50 ppb aflatoxins in wheat and 13.0 ppb aflatoxins in corn. Similar to our results,

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highest value of total aflatoxins found in wheat grain sample (W9) i.e. 8.51 ppb (*Fig. 1*). The concentration of total aflatoxins in maize grains ranged from 14.5 to 92.4 ppb was found in a report conducted in Pakistan (Gillani et al., 2022). While, a study conducted by Sohail et al. (2020) with 120 feed samples collected from the District Mansehra, it was reported that almost 92.5% of samples were contaminated with aflatoxin.

Table 3. *Summary of total aflatoxin contamination in overall samples*

Sample type	No. of samples	Positive samples	%	Mean ± STD (ppb)	Rang (min-max)
Cereals					
Wheat grains	10	10	100	32.06 ± 2.853	0.66-8.51
Corn	10	10	100	31.54 ± 2.256	0.69-7.71
Total	20	20	100	63.60 ± 5.109	0.66-8.51
Pulses					
Red lentils	5	3	60	0.30 ± 0.147	0.07-0.15
Yellow lentils	5	2	40	0.30 ± 0.090	0.09-0.21
Brown lentil	5	4	80	1.18 ± 0.288	0.05-0.53
Black eyed pea	5	2	40	1.20 ± 0.072	0.10-1.10
Brown chickpeas	5	5	100	21.54 ± 0.939	2.42-6.74
Total	25	16	64	24.52 ± 1.536	0.05-6.74
Grand total	45	36	80	88.12 ± 6.645	0.05-8.51

STD = standard deviation

Like Aflatoxins, DON becomes more and more important because of its widespread presence in food items around the world. Acute DON consumption in animals causes malnutrition and diarrhea (Pleadin et al., 2019). In the current study, DON was confirmed in 80% cereals and 92% pulses samples of different varieties. 65% cereals and 76% pulses samples had DON above permissible limit. The concentration of DON in cereals and pulses was ranged 0.07-20.13 ppm and 0.08-22.52 ppm, respectively. Zhang et al. (2023) reported the concentration of deoxynivalenol 9.41-1570.35 ppb in edible and medicinal plants. In Pakistan, Iqbal et al. (2021) reported that 44.2% samples were observed to be contaminated with DON and 7.5% of samples of corn and corn products were contaminated with DON at levels greater than the EU's suggested limits. While, a study conducted by Mruczyk et al. (2021) 110 samples of baby products based on rice, wheat, maize and multigrain available on the western Polish market, it was reported that no high DON content and high estimated daily intake were observed in the analyzed products. In the study conducted with samples of 31 unprocessed wheat and 35 grains of white wheat flour, harvested in 2014 in Romania, the DON levels were determined at 110-1787 μg/kg in 8 wheat samples and 190 μg/kg in one wheat flour sample (Stanciu et al., 2017). Research conducted in Türkiye by Şahin et al. (2023) reported that DON was detected in 4 of the 96 grain cereal samples and highest DON level was found in the corn flour sample, which was sold unpacked. Omurtag et al. 2003) investigated the occurrence of deoxynivalenol (DON) in cereal and pulse products in Turkey. DON was detected in six (8.82%) of 68 cereal and in none of 15 pulse products. The maximum detected amount was 2.67 ppm in a corn flour sample. But in our research DON level is high in pulses samples mostly in brown chickpeas sample (BC4) i.e. 22.52 ppm (*Fig. 2*). Alemayehu et al. 2023) investigated 150 chickpea

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kernels collected from five districts in Ethiopia. Total aflatoxins levels ranged from 2.5 to 31.1 ppb and a mean of 17.4 ppb. DON (6.7%) ranged from 0.2 to 2.9 ppm. However, in current study, level of DON in brown chickpeas is very high ranged from 6.60-22.52 ppm (*Table 4*).

Table 4. *Summary of deoxynivalenol contamination in overall samples*

Sample type	No. of samples	Positive samples	%	Mean ± STD (ppm)	Rang (min-max)
Cereals					
Wheat grains	10	8	80	46.32 ± 2.171	0.07-20.13
Corn	10	10	100	28.57 ± 3.552	0.67-5.26
Total	20	18	80	74.89 ± 5.723	0.07-20.13
Pulses					
Red lentils	5	5	100	11.68 ± 1.145	0.10-5.97
Yellow lentils	5	4	80	16.57 ± 1.132	2.54-6.36
Brown lentil	5	4	80	7.60 ± 0.875	0.08-3.17
Black eyed pea	5	5	100	11.41 ± 1.058	0.50-5.29
Brown chickpeas	5	5	100	67.28 ± 1.044	6.60-22.52
Total	25	23	92	114.54 ± 5.254	0.08-22.52
Grand Total	45	41	91	189.43 ± 10.977	0.07-22.52

STD = standard deviation

Both people and animals may be affected if they consume aflatoxins and DON-contaminated food or feed. They represent a significant and concealed threat to food safety. As a result, decontamination is a popular study issue all over the globe (Yao et al., 2020). In the present study, physical and natural methods were used to detoxify total Aflatoxins and DON in highly contaminated samples. In physical methods, washing treatment gave 25% Aflatoxins and 27% DON reduction. Washing barley and corn three times in distilled water decreased DON concentrations by 65-69% (Trenholm et al., 1992). While research conducted in Pakistan by Zahra et al. 2020) reported that the percentage of deoxynivalenol after washing of corn, rice, and wheat decreased to 25.65, 21.63, and 21.14%, respectively. Heat in excess water showed 36% and 31% reduction in Aflatoxins and DON (*Fig. 3*). Total aflatoxin reduction ranging from 33.6% to 89.9% in boiled maize (Daba et al., 2024). Thermal degradation of AFB1 in foods showed that temperatures higher than 100°C are required to attain at least partial detoxification (Samarajeewa et al., 1990). Cooking showed 46% and 44% effective in aflatoxins and DON reduction. Physical ways of cooking resulted in the highest percentage reduction of deoxynivalenol (39.62%) (Zahra et al., 2020). In natural methods, mustard oil showed 39% and 34% effectiveness for the removal of aflatoxins and DON. Black seed oil showed 52% and 45% reduction and lemon juice showed 64% and 58% effectiveness (*Fig. 4*). Using black seed oil, the greatest decrease level in corn (54.22%) was observed. Aflatoxin reduction in wheat reached its maximum (77.22%) reported by Zahra et al. 2020). A notable reduction in aflatoxins concentration was noted on treatment of infected spices with black seed oil. It is safe to use black seed oil to reduce AFB1 infection (Khosravi et al., 2011). It is claimed that 5% of lemon juice is citric acid (Saidan et al., 2004). In a study, soaking the aflatoxin-contaminated red chilies in lemon juice detoxified them. The findings showed that 90% of the aflatoxin in red

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chilies was decreased by citric acid, demonstrating its high effectiveness against aflatoxin B1. Similarly, another study found that applying citric acid or lemon juice was responsible for 90% of the AFB1 detoxification in pistachio nuts (Rastegar et al., 2017). However, present study showed lemon juice was most effective for the removal of Aflatoxins and DON as compare to mustard oil and black seed oil.

Conclusion

The findings highlight significant contamination of both cereals and pulses by total aflatoxins and DON collected from different areas of Lahore, Pakistan. Overall, 100% cereals and 64% pulses samples are contaminated by total aflatoxins and 80% cereals and 92% pulses samples are contaminated by DON. The wheat grain sample (W9) has the greatest overall aflatoxin level (8.51 ppb), whereas the brown chickpeas sample (BC4) have the highest DON level (22.52 ppm). Also, there is significant difference between total aflatoxins and DON in pulses samples at $p < 0.05$. 10 of 45 samples (22%) exceed the EU regulatory limit for total aflatoxins, while 32 samples (71%) exceed the EU (2023) regulatory limit for DON contaminants. On the other hand, physical detoxification treatments reduce such toxins by 25–46%, whereas natural detoxification treatments reduce them by 34–64%. Thus, using natural methods to lessen the deadly effects of total aflatoxins and DON on individuals and animals may be an appropriate strategy. These detoxification methods are ways to lessen the effects or stop the contamination, but they must be assessed holistically, tested for stakeholder acceptance and consensus to gain support, and in certain situations, tested to produce scientific proof of their efficacy in reducing mycotoxins.

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Declaration of competing interest. The authors declare no conflict of interest.

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