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Effects of temperature and *Saccharomyces cerevisiae* co-culture on mycotoxins stability and decontamination in wheat

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ABSTRACT

BACKGROUND. Mycotoxins found in cereals have adverse effects on animals, humans, and agricultural products, posing significant health risks and affecting the marketability of wheat products.
AIM. This study aimed to identify mycotoxin contamination in durum and bread wheat and to evaluate the effects of heating and *Saccharomyces cerevisiae* co-culture on controlling toxigenic fungal growth and decontaminating mycotoxins in stored wheat products.
MATERIALS AND METHODS. Aflatoxins and ochratoxins were extracted from various samples of durum and bread wheat available in the Pakistani market using methanol, water, and n-hexane. Mycotoxins were identified using thin-layer chromatography (TLC). Fungal species were identified using Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA), with samples incubated at 28°C for 6 to 7 days. The stability of mycotoxins during thermal processing was assessed using traditional cooking methods.
RESULTS. Eight samples with aflatoxins and six with ochratoxins had levels above the European Union Commission's maximum limits. The sample from Faisalabad containing aflatoxin B2 was notably above the maximum threshold. Thermal treatment completely eliminated mycotoxins, enhancing food safety. Additionally, *Saccharomyces cerevisiae* co-culture significantly inhibited mycotoxin growth, making the grains safer and increasing their marketable value.
CONCLUSIONS. Thermal processing effectively eliminates mycotoxins from wheat samples, ensuring food safety. *Saccharomyces cerevisiae* co-culture is a promising method to inhibit mycotoxin growth, thereby improving the safety and marketability of wheat products.

KEYWORDS

MYCOTOXINS
STABILITY
DECONTAMINATION
SACCHAROMYCES CEREVISIAE
WHEAT
THERMAL PROCESSING

INTRODUCTION

Mycotoxins are small molecular weight fungal toxins mainly produced by three different toxigenic fungi, including Fusarium, Aspergillus, and Penicillium. They are generally found on cereals (wheat, corn, barley, etc.), sugar cane, peanuts, rye, etc., and cause a variety of undesirable effects in both animals and humans. It usually grows at 10 to 40°C, from 4 to 8 pH ranges, and at water activity (aw) above 0.70. The term mycotoxins was first used in 1962 after the death of 100,000 turkeys in England[1-3]. The Food and Agriculture Organization (FAO) estimates that mycotoxin contamination is about 25% of the world's agricultural commodities[4].

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The most common mycotoxins are aflatoxins, ochratoxin A, fumonisins, trichothecenes, and zearalenone. Aflatoxins are bisdihydrofurano metabolite, which are synthesized by different strains of toxigenic fungi. Ochratoxins are polyketide and dihydroisocoumarin, which are linked with 12-carboxyl group by an amide bond to L-β-phenylalanine. Trichothecene is a type of mycotoxins called sesquiterpenoids. This naturally occurring toxins have an epoxy group at C-12, 13 and an olefinic bond at C-9, 10. Aflatoxins are powerful carcinogens that cause hepatitis B and are responsible for many deaths. Ochratoxin A is also carcinogenic and causes kidney cancer. Fumonisins cause oesophageal cancer, trichothecenes are highly immunosuppressive, and zeralenone causes estrogenic effects in humans and animals[5-7].

Mycotoxins have different types of toxic effects, i.e., acute, chronic, and teratogenic. The most common is the acute mycotoxins effect, which causes liver and kidney disorders and, in extreme conditions, leads to death. Mycotoxins also interfere with protein synthesis and cause skin sensitivity and immunodeficiency. Some mycotoxins are neurotoxins in nature, which cause continuous trembling in animals in small amounts, while high doses may lead to brain damage and death. Chronic effect of mycotoxins leads to liver cancer. The teratogenic effect of mycotoxins causes mutation in DNA, thus altering cell behavior, and can lead to cancer or other types of abnormalities[8,9].

The FAO appraises that 25% of the world's crops are influenced by mycotoxins every year. The losses due to mycotoxins can be direct and indirect. Direct losses reduce harvest yields, lead to increased livestock death, make surviving animals less productive, increase susceptibility to other diseases, etc. Indirect losses related to the economy increase due to toxigenic fungus control and also expenses associated with the diagnosis and treatment of affected humans and animals. Indirect losses are as critical as the direct effects, in spite of the fact that they are harder to evaluate. For the cultivator, mycotoxin contamination holds down markets, decreases the crop's marketable value, and makes crops not fit for sale[10].

Wheat is the basic and most important global cereal cultivated in the world's subtropical and mild regions. Wheat is used to make food products, e.g., pasta, couscous, bread, noodles, bulgur, etc. The dietary estimation of wheat is critical as it gives around 60% calories. Wheat also contributes fundamental amino acids, minerals, vitamins, valuable phytochemicals and dietary fiber parts to the human eating routine. Wheat is an essential dietary prerequisite in Pakistan. Pakistan is the 8th largest wheat-producing country, supplying approximately 17.3% of the world's wheat11-14. In Pakistan, through plant breeding procedures, researchers have developed numerous varieties of bread and durum wheat, which are consumed by our local population randomly. All these varieties have different potential to resist various types of pest contamination. Also, our unpublished data indicate that many of these varieties are significantly different concerning glycemic index, reactive oxygen species, scavenging potential, and some chemical constituents. Wheat grains are mainly contaminated by different species from Fusarium, leading to Fusarium head blight which is the most toxic disease in all cereals (wheat, barley, oats, rice, etc.). According to a survey conducted by the animal health and nutrition company (BIOMIN) in 2016, wheat samples from 81 different countries were found contaminated with mycotoxins. In Pakistan, the contamination of aflatoxins and the presence of *Aspergillus* species have been reported in some of the bread wheat varieties; however, many of the bread and durum wheat varieties have not been studied for toxigenic fungal growth and mycotoxins contamination[13,15,16].

In developing countries like Pakistan, agricultural export to other countries is affected by mycotoxin contamination, and it also causes increased threats to food safety for the local population. Research advances have led to the development of some techniques that minimize the risk of aflatoxin contamination. These include the application of bio-control agents, bio-degrading agents (microbes and/or their enzymes), and some other physical or chemical treatments during the processing of foods[17,18].

The current research work has been designed to study mycotoxin contamination of both durum and bread wheat and to find out the effects of thermal treatment and *Saccharomyces cerevisiae* co-culture in the control of toxigenic fungal growth and mycotoxins decontamination in the stored wheat products.

MATERIALS AND METHODS

Samples collection

Thirteen different lines of durum wheat and 14 different lines of bread wheat were collected from Agriculture University Peshawar, Cereal Crops Research Institute Pirsabak Nowshera (CCRI), Tarnab Agricultural Institute Peshawar and Nuclear Institute for Food and Agriculture (NIFA). These wheat lines are easily available in the Pakistani market and are used for domestic purposes. Samples were stored at room temperature for 6 months.

Detection of toxigenic fungus

A total of 65 g of Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) were added to 1000 ml distilled water and boiled to dissolve completely. The media was sterilized by autoclaving at 15 psi (121°C) for 15 to 30 minutes and was cooled up to 45-50°C. The media was poured into sterile petri plates. The wheat samples were washed with distilled water and then with 70% ethanol solution to remove the dust and impurities. The grains were cut into two equal fragments to expose the internal tissue for better fungal growth and were placed deep in the media in Petri plates. The plates were incubated at 28°C and, after 6 to 7 days, the fungal colonies were observed through microscopy[19-21].

Extraction and analysis of mycotoxins

Mycotoxins were extracted from durum and bread wheat samples using methanol/water (80:20) and 20 mL of cyclohexane or n-hexane for 3 min. After the separation of the two phases, cyclohexane or n-hexane was eliminated. The aqueous phase was collected and filtered, and various mycotoxins, i.e., aflatoxins (B1, B2, G1, and G2) ochratoxin A, fumonisins, trichothecenes, and zeralenone were identified using Thin Layer Chromatography (TLC)[22-25].

Effect of heating on mycotoxins stability

Wheat samples were grinded to make powder. A total of 200 g powder wheat samples were taken, and 130 ml distilled water was added to make a slurry and was divided into 3 equal portions to make bread. The bread was cooked at different temperatures (100°C, 150°C, and 300°C) using three different traditional cooking methods, i.e., Iron tawa, Oven, and Earth oven. Iron tawa took 8 minutes, the oven took 30 minutes, and the earth oven took 10 minutes to cook bread. To see the effect of temperature on mycotoxins, the bread samples, including their uncooked raw wheat, were ground again for the extraction of aflatoxin and ochratoxin A as per the method mentioned above[5,23,26-29].

Effect of Saccharomyces cerevisiae co-culture on toxigenic fungus and mycotoxins level

Saccharomyces cerevisiae (yeast) was added to a paste of selected varieties of durum and bread wheat flour containing high contamination of mycotoxins-producing fungi. These samples were incubated for various time periods. A control wheat variety was processed without yeast addition[19-21,30]. Mycotoxins were extracted and quantified using Thin Layer Chromatography (TLC)[22-25].

RESULTS

Identification of toxigenic fungus

Mycotoxins are mainly produced by three genera of fungi: Aspergillus, Penicillium, and Fusarium. We sought to determine which species are more prevalent in contaminating our locally grown bread wheat varieties and durum wheat lines. The samples were placed in petri plates with Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) media and incubated at 28°C. After 6 to 7 days, the fungal colonies were observed through microscopy, and the identified toxigenic fungal species are shown in Figure 1.

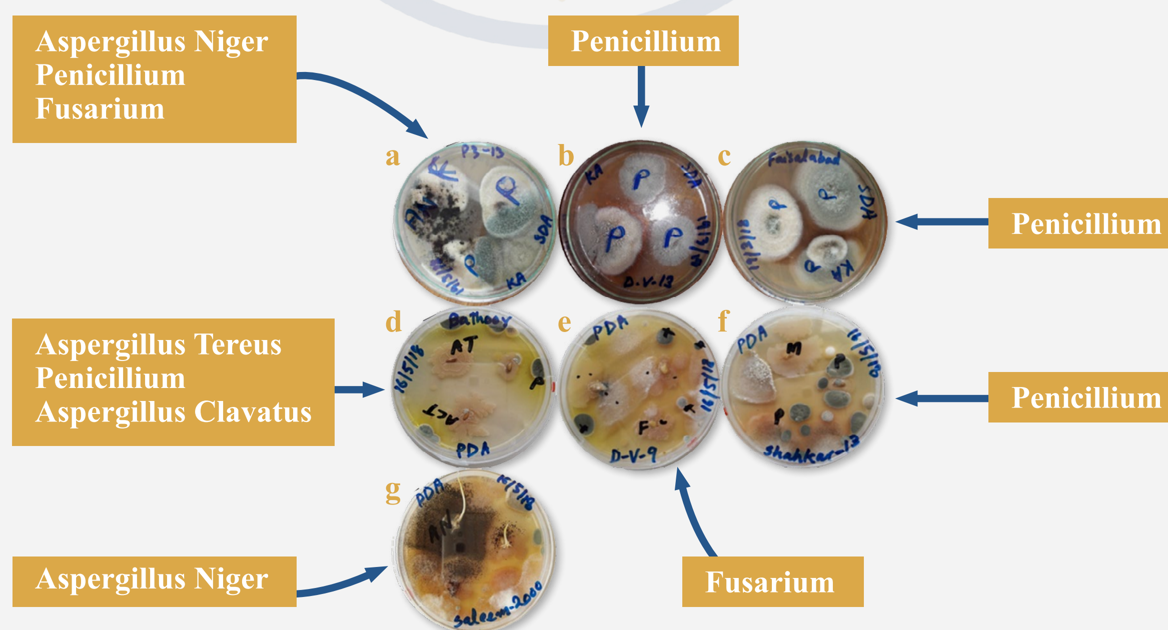

Figure 1. Fungal species identified through microscopy technique, where (a) Pirsabaq-13, (b) Durum Wheat Line-13, (c) Faisalabad, (d) Bathoor, (e) Durum Wheat Line-9, (f) Shakar-13 and (g) Saleem 2000.

Concentration of Aflatoxins and Ochratoxin A in various varieties of wheat

Various durum and bread wheat samples were subjected to mycotoxin extraction (Aflatoxin B1, B2, G1, G2, and Ochratoxin A), and their concentrations were determined. After extracting aflatoxins from 13 durum wheat lines, lines 1, 2, 7, 9, 12, and 13 were found to be contaminated with aflatoxin B1. One sample (Line 5) was found to be contaminated with aflatoxin G1 (7.76 ppb). Out of 14 samples of bread wheat, contamination was observed in Faisalabad, PS-13, Shahkar-13, and Fakhar-e-Sarhad samples. Faisalabad (AFB1 = 4.85 ppb, AFB2 = 16.26 ppb) and PS-13 (AFB1 = 15.77 ppb, AFB2 = 8.49 ppb) samples were contaminated with both aflatoxin B1 and B2. In total, thirteen samples of durum wheat and 14 samples of bread wheat were extracted and evaluated for Ochratoxin A contamination levels. Two samples of durum wheat (Line 1 = 4.40 ppb and Line 3 = 13.65 ppb) and six samples of bread wheat (Faisalabad = 13.21 ppb, Hashim-10 = 8.81 ppb, PS-13 = 6.16 ppb, Saleem 2000 = 11.01 ppb, Barsat = 4.84 ppb, and PS-15 = 14.97 ppb) were found contaminated (Table 1).

Effect of thermal treatment on mycotoxin stability

Various wheat samples, including two bread wheat varieties (PS-13 and Faisalabad) and one durum wheat line (Line 13), were subjected to thermal treatments (100°C, 150°C, and 300°C) following traditional cooking methods (Oven, Iron Tawa, and Tandoor). Mycotoxins were extracted as described previously and analyzed using thin-layer chromatography. The concentration was found in raw unprocessed samples after extraction. No contamination was found in any processed sample at any temperature. The results indicated that cooking wheat flour at high temperatures effectively decontaminated mycotoxins (Table 2).

Effect of Saccharomyces cerevisiae co-culture on fungal growth

Saccharomyces cerevisiae (yeast) is commonly used in making wheat flour paste, which is then kept for a few hours before making roti food items. Previous reports suggest that yeast extract can inhibit some fungal growth. To investigate the effect of yeast on toxigenic fungal growth, we followed a co-culture strategy. The fungal growth was seen in plain culture with mixed grain samples, while no fungal growth was observed in active yeast media (Table 3).

DISCUSSION

Our study confirmed that Aspergillus, Penicillium, and Fusarium are prevalent in contaminating locally grown bread wheat and durum wheat lines. This aligns with previous findings that these genera are major producers of mycotoxins. The identification of these fungi in our samples underscores the need for vigilant monitoring and control measures to ensure food safety.

The detection of aflatoxins and ochratoxin A in several wheat samples highlights the significant contamination risk posed by mycotoxins. Eight out of eleven contaminated samples had levels above the European Union Commission's maximum limits for mycotoxins, indicating a serious health risk31. These findings necessitate stringent quality control and regular testing of wheat products to protect consumers.

Thermal treatment was highly effective in eliminating mycotoxins from wheat samples. This finding is particularly relevant for traditional cooking methods, which often involve high temperatures. Our results suggest that cooking wheat flour at high temperatures can significantly reduce the toxic effects of mycotoxins, thereby enhancing food safety.

The co-culture of Saccharomyces cerevisiae with wheat samples effectively inhibited the growth of toxigenic fungi. This yeast-mediated inhibition presents a safe and cost-effective strategy for controlling fungal contamination in grains. By incorporating yeast in the preparation process, the safety and marketability of wheat products can be improved. This approach also offers a potential method for large-scale application in food processing industries.

CONCLUSIONS

Our study demonstrates the prevalence of mycotoxins in local wheat varieties and the efficacy of thermal treatment and yeast co-culture in mitigating this contamination. These findings can inform national legislation on mycotoxin limits and encourage the adoption of effective control strategies. A comprehensive, large-scale study is recommended to further refine these approaches and ensure precise formulation of safety regulations.

Table 1. Concentration of Aflatoxin and ochratoxin in durum, and bread wheat samples.

	S. No	Type of cereal	Aflatoxin B1 (ppb)	Aflatoxin B2 (ppb)	Aflatoxin G1 (ppb)	Aflatoxin G2 (ppb)	Ochratoxin A (ppb)
Durum Wheat Samples	1	Line 1	12.13	ND	ND	ND	4.40
	2	Line 2	13.34	ND	ND	ND	ND
	3	Line 3	ND	ND	ND	ND	13.65
	4	Line 4	ND	ND	ND	ND	ND
	5	Line 5	ND	ND	7.76	ND	ND
	6	Line 6	ND	ND	ND	ND	ND
	7	Line 7	14.56	ND	ND	ND	ND
	8	Line 8	ND	ND	ND	ND	ND
	9	Line 9	10.92	ND	ND	ND	ND
	10	Line 10	ND	ND	ND	ND	ND
	11	Line 11	ND	ND	ND	ND	ND
	12	Line 12	6.79	ND	ND	ND	ND
	13	Line 13	9.70	ND	ND	ND	ND
Bread Wheat Samples	1	KPK-15	ND	ND	ND	ND	ND
	2	Faisalabad	4.85	16.26	ND	ND	13.21
	3	Hashim-10	ND	ND	ND	ND	8.81
	4	Bathoor	ND	ND	ND	ND	ND
	5	PS-13	15.77	8.49	ND	ND	6.16
	6	Tatara-96	ND	ND	ND	ND	ND
	7	Insaf-15	ND	ND	ND	ND	ND
	8	Amin-10	ND	ND	ND	ND	ND
	9	Shahkar-13	15.29	ND	ND	ND	ND
	10	Saleem 2000	ND	ND	ND	ND	11.01
	11	PS-15	ND	ND	ND	ND	14.97
	12	FS	13.83	ND	ND	ND	ND
	13	PS-2008	ND	ND	ND	ND	ND
	14	Barsat	ND	ND	ND	ND	4.84

ND = Not Detected; ppb = Parts per billion; PS-13 = Pir Sabaq 2013; PS-15 = Pir Sabaq 2015; KPK = Khyber Pakhtunkhwa; FS = Fakhr-e-Sarhad

Table 2. Concentration of aflatoxins and Ochratoxin A in durum wheat and bread wheat after thermal treatment.

S. No	Samples	Raw sample			Oven 100°C Time/conc	Iron Tawa 150°C Time/conc	Tandoor 300°C Time/conc
		AF (B1) (ppb)	AF (B2) (ppb)	Ochra-toxin A			
1	PS-13	15.77	8.49	6.16	30 min/ND	10 min/ND	8 min/ND
2	Durum wheat Line 13	9.70	ND	13.65	26 min/ND	8 min/ND	7 min/ND
3	Faisalabad	4.85	16.26	13.21	32 mint/ND	10 min/ND	8 min/ND

PS-13 = Pirsabaq-2013

Table 3. Result of plain culture with mix grains versus active yeast culture media.

Sample	Name of sample	Species identified	Colony size
Plain culture with mixed grains	Mixed grains	*Penicillium* with traces of *Aspergillus Nigar*	18 mm
		Aspergillus Fumigatus	15.6 mm
Active yeast Culture media	PS 2013	*Penicillium with traces of Aspergillus Nigar*	ND
	PS 2015	*Penicillium with traces of Aspergillus Nigar*	ND
	Durum Wheat line 1	*Aspergillus Fumigatus*	ND

PS 2013 = Pir Sabaq-2013; PS 2015 = Pir Sabaq-2015; ND = Not detected

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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