Research Article

Mycotoxicolgical Evaluation of Indigenous Varieties of Wheat from Quetta, Balochistan, Pakistan.

Shahina Hameed*¹, Nadeem Rashid¹, Farhat Abbas¹, Mustafa Rahim Abro¹, Irfan Shahzad Sheikh¹, Babar Hilal Ahmad Abbasi¹, Roha Talat¹, Samina¹ and Mujahid Farooq²

¹Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta, Pakistan.

²Additional Principal Veterinary Officer, Balochistan Livestock and Dairy Development Department, Quetta, Pakistan.

*Corresponding author: s.siddiqui1234@hotmail.com

Abstract

The present study was designed to investigate the mycological contamination and aflatoxigenic potential of fungi isolated from the indigenous certified varieties of wheat. Surface spread method was used to determine mycological contamination whereas to determine the toxigenic potential of isolated fungi and screening of wheat grains for aflatoxin contamination thin layer chromatography was used. All the collected samples revealed fungal contamination however none of the fungal isolate showed aflatoxigenic potential. Similarly all the samples showed negativity for aflatoxin. It can be concluded that for human public health, cereal grains must be subjected to quality control and mycotoxicolgical examinations.

Keywords: Aflatoxin, Thin Layer Chromatography, Grains, Contamination

ARTICLE INFORMATION

Received: 10.10.2019

Revised: 23.11.2019

Accepted: 31.12.2019

DOI: 10.31580/pjmls.v2i4.1195

© Readers Insight Publication

INTRODUCTION

Wheat (*Triticum aestivum*) is one of the important cereal crop produced and consumed around the world. Pakistan is among the largest wheat producing countries and this grain being a staple diet holds a distinct position by constituting eighty three percent of the total cereal intake (1). It is the cheapest source of energy and protein for the inhabitants of Pakistan contributing more than 60% of the total protein and calorie requirements (2). Wheat provides more nourishment compared to other food grains (3). In Pakistan, the most commonly consumed and least expensive product of wheat is unleavened flat bread locally known as chapatti. Furthermore, wheat is used for various other bakery products like bread, cookies, cakes, buns, pastries etc (4).

Pakistan has high population growth rate and crop improvement programs have always been more yield-oriented and less attention has been paid to the nutritional quality of grain produced. The nutritional quality of wheat generally depends upon; protein and starch contents, its micronutrient composition and certain physicochemical characteristics (5). Toxigenic fungi are widespread in nature and when contaminate grains they may reduce both the yield and the quality of grains. These fungi can attack grains before harvest in the field or after harvest; during storage, processing and transport and can produce mycotoxins (6).

Mycotoxins are structurally diverse, extremely toxic secondary metabolites, produced by molds principally belonging to the genera *Aspergillus*, *Penicillium* and *Fusarium*

(7). Various biological effects like immunosuppression, carcinogenicity, teratogenicity and mutagenicity on humans and animals are proven to be exhibited by mycotoxins. Among mycotoxins, aflatoxins are believed more nitrous. Aflatoxin contamination has been a global issue for human health and animal production (8).

It is a foremost dilemma of developing countries specially located in tropical and subtropical regions; agricultural commodities become contaminated with fungi and consequence aflatoxin formation. Prevalence of aflatoxins in food stuff especially in cereals is reported in the country (9). However; the research on the contamination of wheat with molds and co-occurrence with aflatoxin is still limited in this part of country. This study can further provide the basis for future mycotoxicolgical studies and the development of effective hazard control strategies. Keeping in view the importance of contamination of staple diet with fungi present study was aimed to evaluate indigenous certified varieties of wheat developed in the province for their mycotoxicolgical and physical properties.

MATERIALS AND METHODS

Study Area and Sampling

Samples of different indigenous varieties (n=5) of wheat viz Umeed, Rascho, Zarghoon, Zardana and Zarlashtra developed by Arid Research Institute (ARI) Quetta, Balochistan, Pakistan were collected from ARI, Sariab road Quetta. The wheat samples were collected by hand in sterile polyethylene bags and transferred to Nutrition and toxicology

laborites of Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), Quetta, Balochistan for further processing and analysis.

Processing

The samples were processed for mycological assessment (viable fungal count, isolation of fungi), toxicological analysis (screening for aflatoxin B₁ contents in isolated molds and wheat samples) and physical properties (pH, moisture content) to attain the goal of the study.

Mycological Assessment

Total viable fungal count (TVFC) was carried out using surface spread method previously adopted by (10). Tenfold serial dilutions 10^{-2} – 10^{-4} for each sample (in triplicate) were prepared with sterilized distilled water, spread (100 µl) on the surface of sabouraud dextrose agar (SDA), incubated for seven days in dark at 28°C and the petridishes containing 10-100 colony forming units (CFU) were used to determine TVFC and the results were expressed as CFU per gram of sample. Firing and inoculation technique was used to isolate (Purify) the molds.

Toxicological Analysis

All the isolated molds and collected wheat samples were analyzed to determine aflatoxin B₁ (AfB₁) contents following the methodology of (10). For the purpose fungal isolates were incubated in glass petri dishes containing 25 ml Czapek's solution agar with the same conditions mentioned above. After 10 days incubation period the petri dishes were momentarily autoclaved and the contents were added 75 ml water and then 25 ml chloroform (CHCl₃). The aqueous slurry was blended using tissue homogenizer and the CHCl₃ portion was separated with the help of separating funnel and retained. The chloroform extraction of aqueous slurry was repeated and two chloroform fractions were combined, filtered (Whatman paper filter paper No.1), concentrated with the help of rotary evaporator (steroglass strike 202), made the final volume 4 ml with methanol and transferred to borosilicate vial. This final volume was used to determine aflatoxin B₁ by thin layer chromatography (TLC). For the qualitative determination of AfB₁ with the help of TLC following the guidelines of FDA for aflatoxins all the collected wheat samples were subjected to analysis (12).

For the assessment of physical properties 2 gram sample (replicated thrice) for moisture contents and 50 grams (in triplicate) for pH was evaluated using the same technique as used by (10).

Data Analysis

The data regarding mycotoxicological and physical properties was analyzed using one way analysis of variance (ANOVA) with the help of software SPSS 20 for windows. MS Excel 2010 was used for the processing and tabulation of data.

RESULTS

All the fungal colonies isolated from the samples revealed that no one was positive for Aflatoxin B₁ (Table I). Similarly AfB₁ was not detected in any of the sample (Table II) processed for the determination of AfB₁ contamination level.

Viable fungal count (CFU g⁻¹) of the analyzed samples of certified wheat varieties presented in Table II ranged from 1.0×10^3 (Zarlashta) to 1.8×10^3 (Umeed). The significantly ($P < 0.05$)

higher VFC was noted in Umeed (1.8×10^3) Rascho (1.6×10^3) and Zarghoon (1.6×10^3).

Table 1. Viable Fungal Count (VFC) and occurrence of Aflatoxigenic fungi from indigenous varieties of wheat

SNo	Variety	Range	Mean $\pm$ SD	Aflatoxigenic fungi
1	Umeed	1.5x10 ³ -1.8x10 ³	1.6x10 ³ $\pm$ 153 ^a	ND*
2	Rascho	1.4x10 ³ -1.6x10 ³	1.5x10 ³ $\pm$ 100 ^{ab}	ND
3	Zarghoon	1.5x10 ³ - 1.6x10 ³	1.5x10 ³ $\pm$ 158 ^{ab}	ND
4	Zardana	1.4x10 ³ -1.5x10 ³	1.4x10 ³ $\pm$ 58 ^b	ND
5	Zarlashta	1.0x10 ³ - 1.2x10 ³	1.1x10 ³ $\pm$ 100 ^c	ND

*ND= Not detected

Table 2. Aflatoxin B₁ screening of indigenous varieties of wheat with respect to FDA guidelines and level of contamination

Varieties	Low contamination < 20 µg/kg	Medium contamination 20-50 µg/kg	High contamination > 50 µg/kg
Umeed	ND*	ND	ND
Rascho	ND	ND	ND
Zarghoon	ND	ND	ND
Zardana	ND	ND	ND
Zarlashta	ND	ND	ND

ND=Not detected

Table 3. pH and Moisture contents of Indigenous varieties of wheat

Parameters	Varieties	Mean $\pm$ SD	Minimum	Maximum
pH	Umeed	6.32 $\pm$ 0.01 ^d	6.31	6.33
	Rascho	6.70 $\pm$ 0.02 ^c	6.69	6.73
	Zarghoon	6.80 $\pm$ 0.06 ^d	6.74	6.85
	Zardana	6.85 $\pm$ 0.05 ^b	6.81	6.90
	Zarlashta	6.98 $\pm$ 0.02 ^a	6.96	6.99
Moisture	Umeed	6.81 $\pm$ 0.10 ^{ab}	6.72	6.90
	Rascho	6.71 $\pm$ 0.10 ^{ab}	6.60	6.80
	Zarghoon	6.57 $\pm$ 0.22 ^{ab}	6.63	6.80
	Zardana	6.40 $\pm$ 0.20 ^{bc}	6.20	6.60
	Zarlashta	6.21 $\pm$ 0.19 ^c	6.02	6.40

Moisture contents and pH of indigenous certified varieties of wheat are presented in Table III. The moisture contents revealed significant ($P < 0.05$) difference among varieties. The higher moisture contents (6.81 ± 0.09) were noted in Umeed whereas lowest (6.21 ± 0.19) were observed in Zarlashta. The pH values of analyzed samples ranged from 6.96 to 6.99. The data revealed that the pH value of Zarlashta was significantly higher (6.98) than the rest of varieties ranged from 6.31 (Umeed) to 6.99 (Zarlashta).

DISCUSSION

Pakistan is an agro-livestock based economy and wheat is a major crop and staple diet of the people of country (13). Fungal contamination of agricultural commodities is inevitable both in field and storage conditions (14). Under favourable conditions mycological contamination may result in mycotoxin production (15).

Fungal contamination and VFC of food stuff not only emphasizes the risk of mycotoxins but also it is one of important criterion to evaluate the food hygiene (10). In the present study all of the wheat samples revealed fungal contamination with the moderate values (from 1.0×10^3 to 1.8×10^3 CFU g⁻¹) of VFC. However, this mycological contamination did not exceeded the Good Manufacturing Practices Plus (GMP+, 2010) food quality limits (1×10^4 CFU g⁻¹) regarding mycological quality. Results concerning the fungal contamination of wheat have been reported by Saleemi et al. (2016) which are in partial agreement with the present study. He analyzed 72 wheat samples collected from Faisalabad, Pakistan and noted samples were contaminated with fungi having the potential to produce aflatoxins. Similarly Asghar et al., (2016) analyzed 185 wheat grain samples collected from different areas of Pakistan and reported 50% were contaminated with fungi. Aflatoxin production is mold specific; *Aspergillus flavus* and *Aspergillus paraciticus* are the major producing species whereas *Aspergillus nomis* and – also have the ability to produce aflatoxins. The results of the present study indicate that wheat samples were contaminated with only those fungi not having the potential to produce aflatoxins.

Like fungal isolates also wheat samples showed negativity for AFB₁ during TLC screening. Following the Food and Drug Authority (FDA) and Pakistan standards and Quality Control Authority (PSQCA) total aflatoxins admissible limit (20ppb) for food stuff the samples were found fit for human consumption. Furthermore, following the guidelines of FDA regarding categorizations of food stuff in the present work with a detection limit of 3µg/kg no AFB₁ was detected and the samples were ranked as low contaminated. Similar findings were reported by Najmussahr et al., and Asghar et al., 2016, whom analyzed 60, 185 wheat grain samples collected from different areas of Pakistan and reported aflatoxins were not detected in the permissible limit of 20 ppb.

Moisture content of wheat grains is important in several aspects. It is related to the quality as well as shelf life of grains. High moisture contents (more than 14%) of stored grains are undesirable as they enhance mold growth and other spoiling organisms resulting deterioration in quality (19). The ideal wheat moisture content at harvest should follow between 18% to 20%. This is higher than the perfect wheat moisture content for stored grain. It should bring the moisture content lower to 13.5% if one would plan to sell soon, since that is the acceptable wheat moisture content. If it is planned to store the wheat for one whole year, or more than that, it should be desired to bring it as lower as 12.5%. In the present study moisture contents of different certified wheat varieties were observed below 10% and the findings are in agreement with Khan and Alam, (2007) and Ali et al. (2014) whom reported moisture contents of different indigenous wheat varieties below ten percent. Mean pH value of the analyzed samples revealed slightly acidic most of the fungi grow with in the range 3-7.

CONCLUSION

Mycotoxycological contamination in food stuff occurs due to high moisture content and improper storage temperature. Fungal proliferation and mycotoxin production in edible commodities could be prevented by maintaining specific

moisture content and storage temperature. This study suggests that the staple cereals like wheat should be regularly monitored for mycobiota and mycotoxins.

REFERENCES

1. Shuaib M, Zeb A, Ali Z, Ali W, Ahmad T, Khan I. Characterization of wheat varieties by seed storageprotein electrophoresis. African Journal of Biotechnology. 2007;6(5).
2. Bostan N and Muhammad N. Evaluation of resistance in some wheat cultivars to *Tribolium castaneum* (Herbst) under laboratory conditions. Journal of Plant Sciences. 2002;1(2):95-98.
3. Safdar MN, Khalid N, Muhammad A, Amer M, Saeeda R. Physicochemical quality assessment of wheat grown in different regions of Punjab. Pakistan Journal of Agricultural Research. 2009;8:1773-1778.
4. Mahmood A, Anjum FM, Ur-Rehman S, Ahmed A. . Physical properties of some Pakistani wheat varieties. Pakistan Journal of Food Sciences. 2004;14(3-4):1-9.
5. Zeb A, Zahir A, Ahmad T, Abdumanon A. Physiochemical characteristics of wheat varieties growing in the same and different ecological regions of Pakistan. Pak J Biol Sci. 2006;9(9):1823-8.
6. Baliukoniene V, Bakuits B, Stankevicius H. . Mycological and mycotoxycological evaluation of grain. Annals of Agricultural and Environmental Medicine. 2003;10(2).
7. Shi H, Yu P. Natural Occurrence and Co-Contamination of Twelve Mycotoxins in Industry-Submitted Cool-Season Cereal Grains Grown under a Low Heat Unit Climate Condition. Toxins. 2019;11(3):160.
8. Rashid N, Bajwa MA, Rafeeq M, Tariq MM, Abbas F, Awan MA, Khan MA, Shahzad I, Rehman A, Ahmad Z. Prevalence of aflatoxicosis in broiler chickens in Quetta, Pakistan. Pakistan Journal of Zoology. 2013;1(45):4.
9. Khan SH, Shamsul H, Rozina S, Muhammad AA. . Occurrence of aflatoxin B1 in poultry feed and feed ingredients in Pakistan. International Journal of Agro Veterinary and Medical Sciences. 2011;30-42.
10. Khan SH, Rozina S, Muhammad AA. Occurrence of aflatoxin B1 in poultry feed and feed ingredients in Pakistan. International Journal of Agro Veterinary and Medical Sciences. 2011;30-42.
11. Magnoli C, Miazzi R, Palacio G, Angeletti A, Hallak C, Dalcero A. The mycoflora and toxicity of feedstuffs from a production plant in Cordoba, Argentina. Mycotoxin Research. 2002;1(18):7-22.
12. Nadeem R, Bajwa MA, Mohammad MTK, Tanvir A, Majed R, Zafar A, Awan MA, Ali M, Mustafa MZ, Shafee M, Asadullah, Amanullah K. Occurrence of Aflatoxin B Producing Fungi in Finished Commercial Broiler Feed in Quetta. Pakistan Journal of Zoology. 2017;49(3):1071-1076.
13. Majed R, Nadeem R, Muhammad MT, Rassol BT, AsadUllah, Mustafa Z. Evaluation of Alternatives to Antibiotic Feed Additives in Broiler Production. Pakistan Journal of Zoology. 2017;43(3):1063-1069.

14. El Miniawy HM, Ahmed KA, El-Sanousi AA, Khattab MM. Effect of aflatoxin induced immunosuppression on pathogenesis of H9N2 avian influenza virus. Pak Vet J. 2014;1(34):234-8.
15. Abdallah MF, Girgin G, Baydar T. Occurrence, prevention and limitation of mycotoxins in feeds. Anim Nutr Feed Technol. 2015;1(15):471-90.
16. Saleemi MK, Khan MZ, Khan A, Hameed MR, Khatoon A, Abadin ZU, Hassan ZU. Study of fungi and their toxigenic potential isolated from wheat and wheat bran. Toxin Reviews. 2017;36(1):80-8.
17. Asghar MA, Iqbal J, Ahmed A, Khan MA, Shamsuddin ZA, Jamil K. Development and validation of a high-performance liquid chromatography method with post-column derivatization for the detection of aflatoxins in cereals and grains. Toxicology and industrial health. 2016;32(6):1122.
18. Sahar N, Ahmed M, Parveen Z, Ilyas A, Bhutto A. Screening of mycotoxins in wheat, fruits and vegetables grown in Sindh, Pakistan. Pak J bot. 2009;41(1):337-41.
19. Ali E, Shah SZ, Khan WM, Khan MS, Umar N, Munir I, Ali S, Elahi R. Comparative nutrients analysis of weed-affected and weedy check varieties of wheat. Pakistan Journal of Weed Science Research. 2014;1(20).
20. Ikhtiar K and Alam Z. Nutritional composition of Pakistani wheat varieties. Journal of Zhejiang University Science B. 2007;8(8):555-9.