BioScientific Review (BSR)
Volume 5 Issue 2, 2023
ISSN$_{(P)}$: 2663-4198 ISSN$_{(E)}$: 2663-4201
Homepage: https://journals.umt.edu.pk/index.php/bsr

Article QR

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DOI:	https://doi.org/10.32350/bsr.52.03
History:	Received: August 16, 2022, Revised: December 17, 2022, Accepted: January 4, 2023, Published: June 28, 2023
Citation:	Adrees U, Afzal I, Chaudary AS, Zia R, Ali K, Chaudary HS . Sources and Prevalence of Aflatoxin B1 in different rice paddies of Punjab and Sindh, Pakistan. *BioSci Rev*. 2023;5(2):18–25. https://doi.org/10.32350/bsr.52.03
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Conflict of Interest:	Author(s) declared no conflict of interest

A publication of
The Department of Life Sciences, School of Science
University of Management and Technology, Lahore, Pakistan

Sources and Prevalence of Aflatoxin B1 in Different Rice Paddies of Punjab and Sindh, Pakistan

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ABSTRACT

Rice is a major cash crop used all over world. The hygroscopic nature of rice kernel with warm and fumic conditions is favorable to enhance the growth and development of toxigenic fungi which produce mycotoxins. This study estimates the prevalence of aflatoxin B1 in different rice paddies collected from twelve regions of the Punjab and Sindh provinces of Pakistan. All the samples were analyzed for their phenotypic characteristics (appearance, odor, and grain length). Competitive Enzyme Linked Immuno Sorbent Assay (ELISA) was used to evaluate AFB1, both before and after applying the steam decontamination procedure to detoxify aflatoxin contamination. Cultural assay was used to find the source of aflatoxin. PDA and SDA agar were used to detect aflatoxin producing fungal species. Lactophenol Cotton Blue (LCB) staining was used for microscopic identification of fungal contaminants. Paddies from most regions appeared yellowish in color and odorless with the exception of Hafizabad, Jhang, Sheikhupura, and Gujarat (in Punjab), where they appeared blackish yellow in color and with a pungent smell. According to the results, 43 (61.42%) samples were detected with aflatoxin B1 out of 70 samples, with an average of 15.86±1.7 ug/kg, before treating with seam. After treatment with steam, 7 (10%) samples were detected with aflatoxin B1, with an average of 2.55±1.51 ug/kg. Most regions showed the presence of *P. chrysogenum* with the exception of R.Y. Khan, which showed the presence of *A. niger*. Steam, on average, reduced aflatoxin to 51.42%. The current study indicates that steam is an effective treatment to eradicate aflatoxin at industrial level. New approaches may be explored to target the contaminants in order to ensure food safety.

Keywords: Aflatoxin, Punjab, rice, rice paddies, Sindh, steam, toxigenic fungi

1. INTRODUCTION

Different agricultural crops are used worldwide as staple foods. Among these crops, rice (Oryza sativa) is one of the most important and predominantly consumed grains [1]. The two most populous countries of the world, that is, China and India were the biggest rice producing countries of the world in the year 2011, with a gross production of 202.3 million metric tons and 154.5 million metric tons, respectively [2]. Pakistan is also a major competitor in quality rice production. Its major rice growing areas comprise the eastern belt of the province of Punjab [3].

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Rice can be an ideal and active substrate for those particular fungi that produce mycotoxins [4]. *Aspergillus* and *Penicillium* genera strains are the major sources of mycotoxin production [5].

The supportive agents that cause the above strains to produce aflatoxins are insect infestation, climate of the area where rice is stored, and moisture magnitude, as well as storage circumstances [6]. The mycelium of the molds is the primary site where mycotoxins are primarily produced [7-10]. The toxic and hazardous effects of mycotoxins lead towards the induction of carcinogenic, oestrogenic, teratogenic, hormonal, neurotoxic, and immunologic effects in both human beings and other higher animals [11] The main categories of aflatoxins are B1, B2, G1, G2, M1, and M2. B1 is classified as the primary category of carcinogenic compounds by IARC (International Agency for Research on Cancer) that works under the guidelines set by the World Health Organization (WHO) [12]. European Union (EU) has declared that the threshold level of aflatoxin B1 ranges from 2-4 µg/kg which is safe for human consumption [13] The toxic and hazardous effects produced by mycotoxins in human beings and higher animals are dependent on various factors, such as toxin producing species, intake magnitude, exposure timing, mechanism of the producing action, and metabolism. The intake of food contaminated by mycotoxins may tip towards the induction of carcinogenic, oestrogenic, teratogenic, hormonal, neurotoxic, and immunologic effects in both human beings and other higher animals [14]

Different methodologies have been established to check the presence of aflatoxins, such as thin layer chromatography (TLC), enzyme linked immunoassay (ELISA), and high performance liquid chromatography (HPLC) [15]. ELISA is a formal technique which is advantageous due to its reliability even when implemented on a large number of samples and gives rapid results [16]. This study was conducted to examine the prevalence of aflatoxin B1 in different rice paddies of Pakistan and to find out the effectiveness of commercial treatment to control aflatoxin production in paddies.

2. METHODOLOGY

2.1. Collection of Rice Paddies

A total of 69 rice samples were collected from Badeen (4), Dera Ghazi Khan (3), Gujarat (4), Hafizabad (2), Hyderabad (10), Jang (5), Mandi Buhauddin (7), Multan (3), Phalia (3), Rahim Yar Khan (22), Sheikhupura (3), and Sukkar (3), respectively.

2.2. Preparation of Sample

A total of 500g of rice paddy was collected from each bag of each city. After collection, rice paddies were mixed well for grinding. Homogenized paddies were ground by grinder machine (MX2018-9M21-Vanmay) for 10 minutes.

2.3. Analysis through ELISA

After grinding, paddies were analyzed for AFB1 before and after steam treatment. In ELISA, six known standards are used, provided with R-Biopharm Kit and Bio-Tek, to develop the AFB1 standard curve for estimating aflatoxin in test paddies [17]. Bio-Tek ELISA reader was used for reading. Bio-Tek Gen5™ Reader Control Software efficiently collects data for review and export. Once the reading was taken the paddy was steamed for 2 hours in the dryer chamber of steam plant (DZL1-0.7/1.0/1.25-T-Sitong Boiler). Then, the processes were repeated to take aflatoxin reading for evaluation.

2.4. Analysis through Culturing

SDA and PDA agar was used for fungal growth and to find aflatoxin producing fungi species. Homogenized samples of paddies were inoculated and kept at 30ºC for 5 days.

2.5. Microscopic Identification

Fungal contaminants were confirmed microscopically. For detailed microscopic identification, Lactophenol Cotton Blue (LCB) staining was used. Fungal contaminants were identified microscopically at 10X.

3. RESULTS

The current research was carried out to find the source of aflatoxin contamination and its detection in rice paddies collected from different regions of Punjab and Sindh provinces of Pakistan. Phenotypic appearance based on the color and smell of rice paddies and the length of single grain from each city were taken into consideration. The results of the physical examination of each region are explained briefly in Table 1, which also states the results of the aflatoxin B1 of each city in a pattern of before and after steam treatment. For the identification of fungal isolates, Lactophenol Cotton Blue (LCB) staining was used and observed under 10X. Different types of fungi growth was observed in different cities.

Physical appearance of fungi strains on Potato Dextrose Agar and Sabouraud Dextrose Agar along with their microscopic snaps are depicted in Figure 1. *Aspergillus niger* appeared black in color with black conidial spores having yellow margins. *Aspergillus flavus* appeared yellowish-green in color with yellow conidial spores having green margins. *Penicillium chrysogenum* appeared green in color with yellow margins.

4. DISCUSSION

The normal range of AFB1 set by the EU Commission is 5 µg/kg and for total aflatoxin it is 10 µg/kg [18]. The results indicated that average AFB1 range was 15.86±1.7 µg/kg before steam treatment. A total of 43 (61.42%) samples were detected with aflatoxin B1, out of 70 samples. After treating paddy with steam average, AFB1 range was found to be 2.55±1.51 µg/kg. After treatment with steam, 7 samples (10%) were detected with aflatoxin B1, out of 70 samples. The results indicated that steam must be used as a treatment to eradicate fungal contaminants which produce aflatoxin. The results also showed that 61.42% rice paddies were detected with AFB1, when received at mill before treating with steam. While, after treating with steam, their number was reduced up to 10%. According to the current research, steam reduced aflatoxin to 51.42%.

Different methodologies are used for aflatoxin treatment at different levels. Lai XW et al. [19] estimated the prevalence of aflatoxin contamination in 30 samples of rice from China using HPLC-FLD technique. A total of 24 (80%) samples were polluted with aflatoxin B1. Gamma rays can also be used to reduce aflatoxin. Mohamed NF et al. [20] revealed that treatment with gamma rays is also helpful in detoxifying aflatoxin B1. It reduced AFB1 by 64.7%. Pulse light proved very beneficial in lowering the level of aflatoxin. Wang B et al. [21] showed that the treatment of rice with pulse light also assists in the elimination of AFB1 and AFB2. According to their research, AFB1 was reduced up to 75.0% and AFB2 was reduced up to 39.2% in rough rice. Moisture and temperature were found to be the most common reasons for the growth of fungal contaminants. Due to increase in moisture, there are chances of aflatoxin production [22–29].

Table 1. (a) Phenotypic Appearance of Rice Paddies (b) Quantitative Analysis Before and After Steam Treatment (c) Microscopic Identification of Fungal Isolates using LCB Staining

a) Phenotypic Appearance of Rice Paddies												
City	Baddeen	D.G Khan	Gujarat	Hafizabad	Hyderabad	Jang	Mandi Buhauddin	Multan	Phalia	R.Y Khan	Sheikhupura	Sukkar
Color	yellowish	yellowish	yellowish	blackish yellow	yellowish	yellowish	blackish yellow	blackish yellow	blackish yellow	blackish yellow	blackish yellow	blackish yellow
Smell	odorless	Dusty	Dusty	pungent	odorless	odorless	pungent	pungent	pungent	odorless	odorless	pungent
Grain Length mm	5.75±0.95	6±0.81	5±0.1	5±0.1	5.5±0.70	5.6±0.89	5.28±0.48	5±0.1	5±0.1	5.5±0.67	5±0.1	6±1
b) Quantitative Measurement of Aflatoxin Before and After Steam Treatment												
Before Steam µg/kg	2.37±0.445	4.96±1.24	18.68±0.75	31.72±1.63	3.03±2.10	38.08±1.09	24.97±2.96	4.24±0.94	44.39±0.96	5.53±0.59	9.48±1.74	3.51±1.08
After Steam µg/kg	1.32±0.32	2.24±0.33	1.02±0.02	1.11±0.11	1.58±0.82	2.56±0.66	3.01±0.99	2.61±0.97	5.92±0.78	2.02±0.34	6.35±1.94	1.65±0.36
c) Microscopic Identification of Fungal Isolates with LCB Staining												
A. niger	✓	✓	×	×	×	×	×	×	×	✓	×	✓
P. chrysogenum	×	✓	✓	✓	✓	✓	×	✓	✓	×	×	×
A. flavus	×	×	✓	✓	✓	×	✓	✓	×	×	✓	×

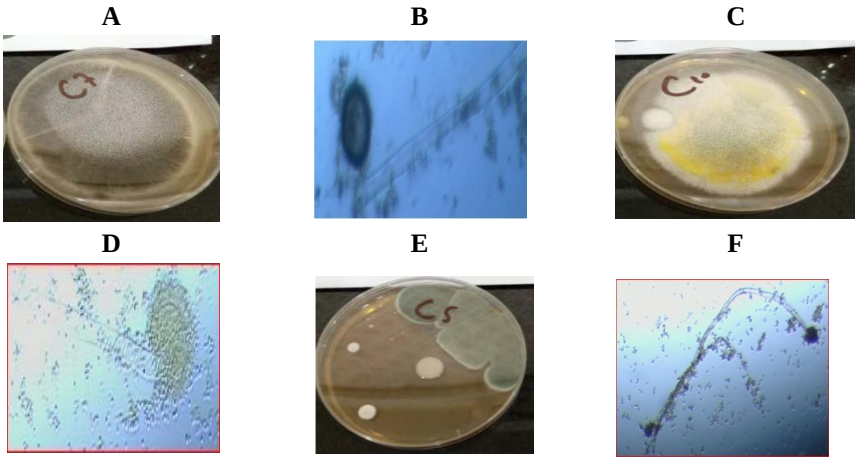

Fig 1. Culture-based Detection of Aflatoxin Producing Fungi. (A, C, E) Fungal Growth of *A. niger*, Fungal Growth of *A. flavus*, Fungal Growth of *P. chrysogenum*. (B, D, F) Microscopic Identification of *A. niger*, Microscopic Identification of *A. flavus*, Microscopic Identification of *P. chrysogenum*

4.1. Conclusion

Steam, on average, reduced aflatoxin by 51.42%. Fungal isolates from most of the regions showed *P. chrysogenum* and *A. flavus*, with the exception of R.Y. Khan which showed *A. niger*. The current study indicated that steam is an effective treatment to eradicate aflatoxin at industrial level.

It also confirmed that rice paddies from Badeen, D.G. Khan, Hyderabad, Multan, R.Y. Khan, and Sukkar have a low level of aflatoxin B1 and are safe to eat. So, it is necessary for regulatory authorities to develop an efficient method for the detoxification of AFB1. They must inspect it on a regular basis. It is also necessary to develop some food quality control procedures and standards, such as good manufacturing practices (GMPs), good agricultural practices (GAPs), and the hazard analysis and critical control point (HACCP) system to minimize the risk of fungal growth and ultimately to prevent the formation of aflatoxins.

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