DOI: https://doi.org/10.54393/df.v5i2.134

DIET FACTOR
Journal of Nutritional & Food Sciences
https://www.dietfactor.com.pk/index.php/df
ISSN (E): 2789-8105, (P): 2789-8091
Volume 5, Issue 2 (April-June 2024)

Original Article

OPEN ACCESS

Determination of Aflatoxin in Various Spices Samples and its Detoxification using Black Seed Oil: A Biological Approach

Muhammad Khalid Saeed[1*], Shaheena Anjum[2], Naseem Zahra[1], Irram Shahzadi[2], Zille Huma[2], Amara Khan[3] and Khalil-ur-Rehman[2]

[1]Food and Biotechnology Research Centre, Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore, Pakistan
[2]Department of Chemistry, Riphah International University, Faisalabad, Pakistan
[3]Institute of Food Science and Nutrition, Gomal University, Dera Ismail Khan, Pakistan

ARTICLE INFO

Keywords:

Aflatoxins, Spices, Detoxification, Biological Approach, Black Seed Oil

How to Cite:

Saeed, M. K., Anjum, S., Zahra, N., Shahzadi, I., Huma, Z., Khan, A., & Rehman, K. ur. (2024). Determination Of Aflatoxin in Various Spices Samples and Its Detoxification Using Black Seed Oil: A Biological Approach: Using Black Seed Oil for Detoxification of Aflatoxins. DIET FACTOR (Journal of Nutritional and Food Sciences), 5(2). https://doi.org/10.54393/df.v5i2.134

***Corresponding Author:**

Muhammad Khalid Saeed
Food and Biotechnology Research Centre, Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore, Pakistan
rosefbrc1@gmail.com

Received Date: 17th May, 2024
Acceptance Date: 27th June, 2024
Published Date: 30th June, 2024

ABSTRACT

Aflatoxins are poisonous compounds generated by specific fungal species that are naturally occurring everywhere and are essentially inevitable. They can seriously endanger human health by contaminating food crops. Aflatoxin contamination of spices is a serious worldwide problem that affects trade and they are cited as the first significant risk in border rejection. **Objectives:** The objectives of this study were to ascertain the aflatoxin content of different spices samples and to use varying concentrations of black seed oil to detoxify positive samples. **Methods:** Thin Layer Chromatography (TLC) was used to determine the aflatoxins in various spices and contaminated sample were detoxified by black seed oil. **Results:** From this study aflatoxins were detected in 70% and 30% spices samples have no aflatoxin. Among contaminated samples 43% had aflatoxins beyond the permissible limits whereas 57% had the aflatoxins below the permissible limits. The positive samples were alleviated by biological method i.e. black seed oil (1-10%) which detoxified aflatoxin in fennel 49.52 ± 1.50 – 92.50 ± 1.94% and detoxification level was found in cumin seed 55.37 ± 1.52 – 87.32 ± 1.83 while in fenugreek it was ranging from 50.20 ± 1.50 – 82.37 ± 1.75. **Conclusions:** This study showed that black seed lowered aflatoxin levels in some spices.

INTRODUCTION

Some types of mould in grains, nuts, spices, and dried fruits contain mycotoxins, which are harmful fungi metabolites. About 300 distinct fungus species, including *Claviceps, Fusarium, Aspergillus, Alternaria*, and *Penicillium*, are known to produce mycotoxins on various substrates due to inappropriate moisture content and temperature. Animals that eat feed tainted with mycotoxin may develop problems with growth, reproduction, or possibly pass away. The majority of these mycotoxins, which are chemically stable and dangerous to humans and animals, are aflatoxins, ochratoxins, zearalenone, nivalenol, deoxynivalenol, citrinin, fumonisins, and patulin, among other strong toxic mycotoxins[1-3]. Mycotoxins have harmful properties such as being immunosuppressive, mutagenic, carcinogenic, teratogenic, and toxic [4]. These genotoxic substances affect the kidneys, liver and immune systems, among other organs. Liver necrosis, anorexia, vomiting, diarrhea and fatty liver are some of their symptoms. Their effects on the reproductive system include a reduction in the percentage of viable sperm, delayed testicular growth, and a drop in testosterone plasma concentration and reduced resistance to bacterial, fungal, parasitic

DOI: https://doi.org/10.54393/df.v5i2.134

andencephalopathy and interstitial fibrosis are two additional associated symptoms [5, 6]. Furthermore, because mycotoxins are frequently combined in food, humans are exposed to many mycotoxins at once [7]. They have the potential to contaminate a broad variety of agricultural and food items, such as grains, oilseeds, spices, tree nuts, and dairy products [8]. Fungal species including Aspergillus flavus, A. parasiticus, and A. specific create aflatoxin, a low-molecular-weight secondary metabolite that is extremely poisonous, carcinogenic, and mutagenic. These fungi are most commonly found in hot, humid climates and are known to produce aflatoxin in food and feed. They are present in soil, plants, animals, and many types of nuts. They are especially prone to infect grains, including wheat, soybeans, rice, corn, peanuts, spices, and oil seeds [9]. Spices have long been known to have therapeutic properties, and research on their capacity to transfer biological activity is gradually making a comeback in the field of human health [10]. Due primarily to their phenolic content, these spices have already been employed as raw materials for a wide range of industries (such as the food, pharmaceutical and cosmetic sectors) as flavorings, food colorings, essential oils, sweeteners, and even for their nutraceutical properties. Spices are becoming more and more popular for their creative application as a source of these naturally occurring bioactive chemicals, along with the growing trend towards the consumption of more natural and ecologically friendly meals [11]. The optimal parameters for the development of fungi, and ultimately the production of aflatoxin, are 25–33 °C, 16–30% moisture content, and 80–100% relative humidity [12]. If the spices are subjected to a fungal contamination during the production process, marketing, post-harvest procedures, farming and processing they are hazards and regrettably, a lot of spices are prone to aflatoxins contamination and are highly vulnerable to toxic fungal strains [13]. A number of techniques have been used to stop the synthesis of aflatoxins or to eliminate, inactivate or reduce their bioavailability in contaminated foods. Aflatoxin detoxification can be achieved by physical (UV light, heat, or ionizing radiation), chemical (adding hydrolytic, chlorinating, or oxidizing agents), or biological techniques [14, 15]. However, due to drawbacks of physical and chemical techniques including the loss of nutritional value, the need for costly equipment as well as their detrimental impacts on humans is rising [16, 17]. This is claimed to be safe, non-pathogenic for human and to maintain the nutritional value of food [18, 19].Biological control is an emerging approach for the degradation of toxins with no threats to health or food material and can significantly reduce 20-90% of infections [20]. *Nigella* sativa, also known as black seed or kalonji, is a member of the *Ranunculaceae* family and is considered lucky to have several biologically active compounds that have been shown to have a range of medical benefits, including antibacterial, immune-modulating, antioxidant, and anti-inflammatory effects [21]. Black seed oil's antifungal properties show considerable potential for *A. flavus* prevention andits oil's minimum inhibitory concentration value is significantly lower than that of other essential oils, making it economically viable for the suppression of food-borne fungi and the biological treatment [22].

The main objectives of this study was to determine the aflatoxin in various spices samples that were collected from the local market and to detoxify highly positive sample using a biological approach i.e. varying concentrations of black seed oil.

METHODS

Collection of Spice Samples

This study was done in Food and Biotechnology Research Centre, PCSIR Laboratories Complex's in Lahore. A total ten spice samples (red pepper, turmeric clove fennel cumin seed fenugreek black pepper cinnamon garlic and ginger) were collected from local market, Lahore. After identification, the entire spices were ground using an electric grinder and stored in a polythene bag for further study.

Aflatoxins Extraction and Analysis

Aflatoxin extraction from spices was done by using the AOAC (2023) technique [23]. Briefly 50g sample of each spices were put in a conical flask and mix it with 200 ml of solvent (aceto-nitrile: water, 9:1). Shake it in an orbital shaker for half an hour at room temperature. Whatman filter paper No. 4 was used to filter the extract. Then the filtrate was evaporated in a rotary evaporator and residue was kept for further analysis. This residue was redisolved in known concentration of chloroform for TLC.

Thin Layer Chromatography (TLC)

Using a micro syringe, samples 5, 10, 15, 20, and 25µl were spotted on a TLC plate at a distance of almost 1.5 cm from the base. In a similar manner, standard spots of 1, 2.5, and 5µl were completed. Following spotting, the plate was placed in the tank which containing a mobile phase anhydrous diethyl ether and was developed, dried and then added to the second tank, which had acetone-chloroform (1:9, v/v) as a second mobile phase. The plate was examined at 365 nm UV light to decide whether aflatoxins were present or not. The sample color and Rf values correspond to the aflatoxin standard [24].

Calculations:

The following formula was used to calculate the aflatoxin concentration:

Contents of aflatoxins (µg/kg) = S×Y×V / W× Z

Where:

S = Aflatoxin standard volume in µl

DOI: https://doi.org/10.54393/df.v5i2.134

Y= Aflatoxin concentration in mg/ml of the reference standard
Z = The volume of sample extract (µl) needed to produce the desired level of fluorescence to that of S = ml of aflatoxins standard which was determined under UV.
W = Effective Weight, in gram, of original sample contained in final extract
V = Volume, in ml, of solvents (chloroform), needed to dilute final extract

Detoxification by Biological Method

To detoxify a sample contaminated with aflatoxin, black seed oil was utilized which was extracted from black seed by soxhlet apparatus. Fifteen gram of contaminated fennel sample were combined with 100 ml of 1%, 2%, 3%, 5%, and 10% black seed oil in a fume hood and left for six hours at 25 ºC. After being shook for three minutes, it was filtered and then redissolved in aceto-nitrile to be spotted on a TLC plate for aflatoxin analysis.

Statistical Analysis

The trials were conducted in triplicate (n=3), and the data was presented as mean ± SD. ANOVA techniques were used for analysis of variance.

RESULTS

Ten samples were chosen at random from Lahore's surrounding market. Aflatoxin B1 was quantitatively examined utilizing the Thin Layer Chromatography (TLC) method. Three of the ten local spices samples were found to be free of aflatoxin contamination, whereas the remaining seven samples were found to be contaminated with aflatoxin B1. Four samples had aflatoxin contamination levels below acceptable limits and three samples had contamination levels above acceptable limits as determined by the European Commission's allowed limit of aflatoxin contamination (10 ppb) for spices and (50 ppb) for red pepper. Aflatoxin B2, G1 and G2 were not detected in any spices sample. The following table 1 displays the aflatoxins' TLC results.

Table 1: Detection of Aflatoxin in Various Spices Samples for by TLC

Sample ID	Samples Names	Aflatoxins (ppb)				Permissible (EU limits)	Status
		B1	B2	G1	G2		
1	Red pepper	40.82 ± 1.38	ND	ND	ND	50 ppb	Fit
2	Turmeric	6.27 ± 0.08	ND	ND	ND	10 ppb	Fit
3	Clove	ND	ND	ND	ND	10 ppb	Fit
4	Fennel	70.50 ± 1.6	ND	ND	ND	10 ppb	Unfit
5	Cumin Seed	9.86 ± 0.12	ND	ND	ND	10 ppb	Unfit
6	Fenugreek	20.42 ± 0.42	ND	ND	ND	10 ppb	Unfit
7	Black Pepper	ND	ND	ND	ND	10 ppb	Fit
8	Cinnamon	ND	ND	ND	ND	10 ppb	Fit
9	Garlic	2.17 ± 0.06	ND	ND	ND	10 ppb	Fit
10	Ginger	8.68 ± 0.10	ND	ND	ND	10 ppb	Fit

*ND means Not Detected

In this study aflatoxin contaminated samples were decontaminated using a biological approach i.e. different concentrations of black seed oil (1–10%) and the outcomes demonstrated that every concentration in the harmful sample of fennel, ranging from 49.52 ± 1.50 – 92.50 ± 1.94%, eliminated aflatoxin. These findings showed that 10% black seed oil was the most successful treatment, reducing AFB1 by up to 92.50 ± 1.94% (Table 2) in fennel sample.

Table 2: Detoxification of Aflatoxins in Fennel Sample by Black Seed Oil

Concentration of Black Seed Oil for Detoxification of AF	Initial Levels (ppb)	Levels after Detoxification (ppb)	Reduction (%)
Black Seed Oil (1%)	70.50 ± 1.60	35.61 ± 1.32	49.52 ± 1.50
Black Seed Oil (2%)	70.50 ± 1.60	28.21 ± 1.02	60.03 ± 1.55
Black Seed Oil (3%)	70.50 ± 1.60	20.45 ± 0.40	71.01 ± 1.62
Black Seed Oil (5%)	70.50 ± 1.60	7.92 ± 0.08	88.76 ± 1.84
Black Seed Oil (10%)	70.50 ± 1.60	5.29 ± 0.06	92.50 ± 1.94

This treatment was also done with the other contaminated samples (cumin seed and fenugreek) and detoxification level was found in cumin seed 55.37 ± 1.52 – 87.32 ± 1.83% (Table 3) while in fenugreek it was ranging from 50.20 ± 1.50 to 82.37 ± 1.75% (Table 4).

Table 3: Detoxification of Aflatoxins in Cumin Seed by Black Seed Oil

Concentration of Black Seed Oil for Detoxification of AF	Initial Levels (ppb)	Levels after Detoxification (ppb)	Reduction (%)
Black Seed Oil (1%)	9.86 ± 0.12	4.40 ± 0.086	55.37 ± 1.52
Black Seed Oil (2%)	9.86 ± 0.12	3.82 ± 0.06	61.25 ± 1.56
Black Seed Oil (3%)	9.86 ± 0.12	3.04 ± 0.04	69.17 ± 1.60
Black Seed Oil (5%)	9.86 ± 0.12	2. 18 ± 0.02	77.89 ± 1.75
Black Seed Oil (10%)	9.86 ± 0.12	1.25 ± 0.01	87.32 ± 1.83

Table 4: Detoxification of Aflatoxins in Fenugreek by Black Seed Oil

Concentration of Black Seed Oil for Detoxification of AF	Initial Levels (ppb)	Levels after Detoxification (ppb)	Reduction (%)
Black Seed Oil (1%)	20.42 ± 0.42	10.15 ± 1.32	50.20 ± 1.50
Black Seed Oil (2%)	20.42 ± 0.42	8.50 ± 1.02	58.37 ± 1.53
Black Seed Oil (3%)	20.42 ± 0.42	6.75 ± 0.40	66.94 ± 1.58
Black Seed Oil (5%)	20.42 ± 0.42	5. 02 ± 0.08	75.41 ± 1.67
Black Seed Oil (10%)	20.42 ± 0.42	3.60 ± 0.06	82.37 ± 1.75

DISCUSSION

Thin layer chromatography is still used for both qualitative and quantitative mycotoxin analysis. The primary reasons for this are the low operating costs, the large sample throughput, and the simplicity of target compound identification utilizing UV-Vis spectral analysis [25, 26]. TLC techniques were employed in a number of investigations to quantify the quantities of aflatoxins (B1, B2, G1 and G2) in spice samples. Hussain *et al.*, in 2023 employed the TLC to determine the amount of aflatoxins in feeds [27]. This TLC method is widely used and highly

DOI: https://doi.org/10.54393/df.v5i2.134

beneficial for determining the aflatoxin levels in spices [28]. Mycotoxins are dangerous and thermo-stable secondary metabolites of fungi that can penetrate food and feed and withstand a range of food microbiological stabilization techniques, such as heating [29]. Consequently, contaminated food and feed exposes humans and animals to negative effects. These can appear on a wide range of foods, such as grains, crops, nuts, fruits, and dried fruits, cheese, and spices, at any point during storage, harvesting and production [30]. Among mycotoxins, aflatoxins are the most dangerous. Currently, a variety of methods (physical, chemical, and biological) are used to detoxify and decontaminate aflatoxins from food and feed [31]. The industry does not use physical or chemical methods due to their high cost, negative effects on texture and taste, and reduction in nutritional value [32]. The majority of researchers have determined that biological methods are the most effective means of decontaminating aflatoxins [33]. Additionally, it was reported that biological methods have been deemed the most effective due to their high efficiency, low cost, eco-friendliness and ability to maintain nutritional quality, when compared to physical and chemical, methods used to prevent the production, reduction, elimination and deactivation of aflatoxin in contaminated food [34]. By using biological techniques for the detoxification of aflatoxins it may not alter the organolaptic properties of food items [35]. Mycotoxins can be absorbed by living or dead microorganisms and stored in their bodies or on their cell walls. As a result of degradation, extracellular or intracellular enzymes can carry out enzymatic degradation. Enzymatic alterations have the ability to alter, diminish, or eliminate toxicity in this manner [36]. It is also believed that moulds that produce aflatoxins break them down through the action of the peroxidase enzyme in the mould mycelia. Aflatoxin reaction with free radicals occurred due to the breakdown of hydrogen peroxides, which is catalyzed by peroxidase. In the presence of hydrogen peroxide and chloride ions, certain peroxidases, such myeloperoxidase, generate hypochlorite and singlet oxygen which efficiently eliminates aflatoxins [37]. In 2021, Nazir *et al.*, also utilize the black seed oil for detoxification of aflatoxin in rice and feed samples and detoxification level was found to 63–100% [38]. In another investigation, black seed oil was applied to contaminated wheat samples which decreased the aflatoxin level up to 81-87% [39]. Black seed oil was used to treat tainted spices in order to detoxify aflatoxin B1. It was discovered that this oil was incredibly effective, reducing contamination levels by 92%. In the black seed oil numerous bioactive compounds and antioxidants found when it was treated with aflatoxin contaminated samples, these bioactive compound showed the inhibitory effects that may decrease the aflatoxin levels. Moreover this oil is well-known for its antifungal properties against a wide variety of fungus. For instance, reported that black seed oil was effective at 0.15%, completely inhibiting *F. moniliforme* and *A. alternata* at doses of 0.1% and 0.15%. [40–42].

CONCLUSIONS

This study reveals that the biological method using 10% black seed oil for detoxification of aflatoxin in spices is very effective.

Authors Contribution

Conceptualization: MKS
Methodology: SA
Formal analysis: NZ, ZH, AM, KR
Writing-review and editing: MKS, IS

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Lai Y, Sun M, He Y, Lei J, Han Y, Wu Y *et al.* Mycotoxins binder supplementation alleviates aflatoxin B1 toxic effects on the immune response and intestinal barrier function in broilers. Poultry Science. 2022; 101(3): 101683. Doi Mar: 10.1016/j.psj.2021.101683.

[2] Qing H, Huang S, Zhan K, Zhao L, Zhang J, Ji C *et al.* Combined toxicity evaluation of ochratoxin A and aflatoxin B1 on kidney and liver injury, immune inflammation, and gut microbiota alteration through pair-feeding pullet model. Frontiers in Immunology. 2022 Jul; 13: 920147. doi: 10.3389/fimmu.2022.920147.

[3] Awuchi CG, Nwozo OS, Aja PM, Odongo GA. High-pressure acidified steaming with varied citric acid dosing can successfully detoxify mycotoxins. Food Science & Nutrition. 2023 Mar; 11: 2677–2685. doi: 10.1002/fsn3.3324.

[4] Mottaghianpour E, Nazari F, Mehrasbi MR, Hosseini MJ. Occurrence of aflatoxin B1 in baby foods marketed in Iran. Journal of the Science of Food and Agriculture. 2017 Jul; 97(9): 2690-4. doi: 10.1002/jsfa.8092.

[5] Ehsani A, Barani A, Nasiri Z. Occurrence of aflatoxin B1 contamination in dairy cows feed in Iran. Toxin Reviews. 2016 Jan; 35(1-2): 54-7. doi: 10.3109/15569543.2016.1155622.

[6] Zolfaghari H, Khezerlou A, Ehsani A, Khosroushahi A Y. Detoxification of Aflatoxin B1 by Probiotic Yeasts

DOI: https://doi.org/10.54393/df.v5i2.134

and Bacteria Isolated From Dairy Products of Iran. Advanced Pharmaceutical Bulletin. 2020 May; 10(3): 482-487. doi: 10.34172/apb.2020.060.

[7] Battilani P, Palumbo R, Giorni P, Dallasta, C, Dellafiora L, Gkrillas A *et al.* Mycotoxin mixtures in food and feed: holistic, innovative, flexible risk assessment modelling approach. EFSA Supporting Publications. 2020 Dec; 17(1): 1-5. doi: 10.2903/sp.efsa.2020.en-1757.

[8] Garda-Buffon J, de Oliveira FK, da Silva JL, Nogueira WV, Badiale-Furlong E. Mycotoxin Degradation Methods in Food. In: Food Safety. CRC Press; 2024. p. 165-182.

[9] Wang Y, Jiang L, Zhang Y, Ran R, Meng X, Liu S. Research advances in the degradation ofaflatoxin by lactic acid bacteria. Journal of Venomous Animal and Toxins include Tropical Diseases. 2023 Oct; 29: e20230029: 1-11. doi: 10.1590/1678-9199-JVATITD-2023-0029.

[10] Mathpal D and Rathore G. A review on health benefits of indian spices. International Journal of Innovative Research in Engineering & Management. 2022 Feb; 9(1): 98-101. doi: 10.55524/ijirem.2022.9.1.16.

[11] Duque-Soto C, Ruiz-Vargas A, Rueda-Robles A, Quirantes-PinéR, Borrás-Linares I, Lozano-Sánchez J. Bioactive potential of aqueous phenolic extracts of spices for their use in the food industry a systematic review. Foods. 2023 Aug; 12:3031. doi: 10.3390/foods12163031.

[12] Abrehame S, Manoj VR, Hailu M, Chen YY, Lin YC, Chen YP. Aflatoxins: source, detection, clinical features and prevention. Processes. 2023 Jan; 11: 204. doi: 10.3390/pr11010204.

[13] Abrar M, Ahsan T, Nadeem M, Liaqat A, Ghugtai MFJ, Farooq MA *et al.* Detection and quantification of aflatoxins in spices stored in different food packaging materials. Journal of Stored Products Research. 2023 Mar; 101: 102081. doi: 10.1016/j.jspr.2023.102081.

[14] Rao KR, Vipin AV, Hariprasad P, Appaiah KA, Venkateswaran G. Biological detoxification of aflatoxin B1 by Bacillus licheniformis CFR1. Food Control. 2017 Jan; 71: 234-41. doi: 10.1016/j.foodcont.2016.06.040.

[15] Afsharmanesh H, Perez-Garcia A, Zeriouh H, Ahmadzadeh M, Romero D. Aflatoxin degradation by Bacillus subtilis UTB1 is based on production of an oxidoreductase involved in bacilysin biosynthesis. Food Control. 2018 Dec; 94: 48-55. doi: 10.1016/j.foodcont.2018.03.002.

[16] An NN, Shang N, Zhao X, Tie XY, Guo WB, Li D *et al.*. Occurrence, regulation, and emerging detoxification techniques of aflatoxins in maize: a review. Food Reviews International. 2024 Jan; 40(1): 92-114. doi: 10.1080/87559129.2022.2158339

[17] Daou R, Joubrane K, Maroun RG, Khabbaz LR, Ismail A, Khoury A. Mycotoxins: Factors influencing production and control strategies. AIMS Agriculture and Food. 2021; 6(1): 416–447. doi: 10.3934/agrfood.2021025.

[18] Perczak A, Golinski P, Bryła M, Waskiewicz A. The efficiency of lactic acid bacteria against pathogenic fungi and mycotoxins. Arh Hig Rada Toksikol. 2018 Mar; 69(1): 32–45. doi: 10.2478/aiht-2018-69-3051.

[19] Muhialdin BJ, Saari N, Hussin AS. Review on the Biological Detoxification of Mycotoxins Using Lactic Acid Bacteria to Enhance the Sustainability of Foods Supply. Molecules. 2020 Jun; 25(11): 1-6. doi: 10.3390/molecules25112655.

[20] Loi M, Logrieco AF, Pusztahelyi T, Leiter É, Hornok L, Pócsi I. Advanced mycotoxin control and decontamination techniques in view of an increased aflatoxin risk in Europe due to climate change. Frontiers in Microbiology. 2023 Jan; 13: 1-5. doi: 10.3389/fmicb.2022.1085891.

[21] ZahraN, SaeedMK, RashidF, SaleemL. Kalonji (Nigella sativa): Examining the features and medical benefits of natural traditional medicine. Chemistry International. 2024 Jan; 10(1): 16-21. doi: 10.5281/zenodo.11000928.

[22] Marrez DA, Shahy EM, El-Sayed HS, Sultan YY. Detoxification of Aflatoxin B1 in milk using lactic acid bacteria. Journal of Biological Sciences. 2018 Mar; 18(3): 144-51. doi: 10.3923/jbs.2018.144.151.

[23] AOAC. Official methods of analysis of the association of official analytical chemists. 21st Edition. USA: Association of Official Analytical Chemists; 2023.

[24] Naseem Z, Imran K, Muhammad KS, Ijaz A. Quality Assessment of aflatoxins contamination in red chillies. Journal of Biotechnology and Bioresearch. 2020 Jan; 2(3). doi:10.31031/JBB.2020.02.000537.

[25] Zahra N, Khan M, Mehmood Z, Saeed MK, Kalim I, Ahmad I *et al.* Determination of aflatoxins in spices and dried fruits. Journal of Scientific Research. 2018 Mar; 10(3): 315-321. doi: 10.3329/jsr.v10i3.37075.

[26] Abrahem SA. Analysing aflatoxin production conditions in feed samples using a preparative thin layer chromatography (TLC) Method. Iraqi Journal of Science. 2022 Jan; 63(1): 9-20. doi: 10.24996/ijs.2022.63.1.2.

[27] Hussain A, Yasmeen R, Hafeez, Saeed K. Monitoring of aflatoxins in broiler, quail and ostrich feed samples. Scientific Reports in Life Sciences. 2023

DOI: https://doi.org/10.54393/df.v5i2.134

Jan; 4(2): 11-23. doi: 10.5281/zenodo.8250942.

[28] Zahra N, Naeem N, Aaliya Iqbal Butt AI, Saeed MK *et al.* Determination of aflatoxins in different varieties of chillies collected from Lahore, Pakistan. International Journal of Food Science and Agriculture. 2022 Sep; 6(3): 349-354.doi:10.26855/ijfsa.2022.09.017.

[29] Conte G, Fontanelli M, Galli, F, Cotrozzi L, Pagni L, Pellegrini E. Mycotoxins in feed and food and the role of ozone in their detoxification and degradation: an update. Toxins. 2020 Jul; 8: 486. doi: 10. 3390/ toxin s1208 0486.

[30] Patel KH, Kalaria RK, Kahimani MR, Shah GS, Dholakiya BZ. Prevention and control of mycotoxins for food safety and security of human andanimal feed. In: Fungi bio-prospects in sustainable agriculture, environment and Nano-technology. Academic Press. 2021 Jan; 3:315–345. doi: 10. 1016/ B978-0- 12- 821734 -4.

[31] Sipos P, Peles F, Brassó DL, Béri B, Pusztahelyi T, Pócsi I *et al.* Physical and chemical methods for reduction in aflatoxin content of feed and food. Toxins. 2021 Mar; 13(3): 1-7. doi: 10.3390/toxins130302 04

[32] Deng LZ, Mujumdar AS, Zhang Q, Yang XH, Wang J, Zheng ZA *et al.* Chemical and physical pretreatments of fruits and vegetables: Effects on drying characteristics and quality attributes. Critical Reviews in Food Science and Nutrition. 2019 Mar; 59(9): 1408-1432. doi: 10.1080/10408398.2017.140 9192.

[33] Marshall H, Meneely JP, Quinn B, Zhao Y, Bourke P, Gilmore BF *et al.* Novel decontamination approaches and their potential application for post-harvest aflatoxin control. Trends in Food Science and Technology. 2020; 106: 489-496. doi: 10.1016/j.tifs.20 20.11.001.

[34] Zolfaghari H, Khezerlou A, Banihashemi SA, Tavassoli M, Ehsani A. Review on bio-detoxification of aflatoxins based on lactic acid bacteria: mechanism and applications. Journal of Microbiology Biotechnology and Food Sciences. 2023 Mar; 13(1): 1-7. doi: 10.55251/jmbfs.9424.

[35] Liu L, Xie M, Wei D. Biological detoxification of mycotoxins: current status and future advances. International Journal of Molecular Sciences. 2022 Jan; 23: 10641071. doi: 10.3390/ijms23031064.

[36] Nasrollahzadeh A, Mokhtari S, Khomeiri M, Saris P. Mycotoxin detoxification of food by lacticacid bacteria. International Journal of Food Contamination. 2022 Jan; 9(1): 1-9. doi: 10.1186/s4055 0-021-00087-w.

[37] Vankayalapati V K. Aflatoxins: properties, toxicity and detoxification. Nutrition and Food Science International Journal. 2018 May; 6(5): 555696. doi: 10.19080/NFSIJ.2018.06.555696.

[38] Nazir A, Kalim I, Imran M, Bilal M A, Zahra N *et al.* Incidences and bio-detoxification of aflatoxins in rice and cattle feed crops under different agro-ecological zones. Polish Journal of Environmental Studies. 2021 Dec; 30(2): 1949-1954. doi: 10.15244/pjoes/121050.
39

[39] Ismail AM, Raza MH, Zahra N, Ahmad R, Sajjad Y, Khan SA. Aflatoxins in Wheat Grains: Detection and Detoxification through Chemical, Physical, and Biological Means. Life 2024 Apr; 14: 535. doi: 10.3390/life14040535

[40] Khosravi A R, Minooeianhaghighi M H, Shokri H, Emami S A, Alavi S M, Asili J. The potential inhibitory effect of Cuminum cyminum, Ziziphora clinopodioides, and Nigella sativa essential oils on the growth of Aspergillus fumigatus and Aspergillus flavus. Brazilian Journal of Microbiology. 2011 Jan; 42(1):216–224. doi: 10.1590/S1517-838220110001000 27.

[41] Saladino F, Luz C, Manyes L, Fernández-Franzón M, Meca G. In vitro antifungal activity of lactic acid bacteria against mycotoxigenic fungi and their application in loaf breads shelf-life improvement. Food Control. 2016 Sep; 67: 273–277. doi: 10. 1016/j.foo dcont.2016.03.012.

[42] Naeem HA, Ashraf A, Rafi U, Zahra N. Microbiological evaluation of different types of branded and non-branded ready-to-eat snacks sold in elementary schools of district Peshawar, Pakistan. BioScientific Review. 2023 Jun; 5(3): 01–14. doi: 10.32350/BSR.53.0 1.