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Determination and detoxification of ochratoxin-A in maize by eco-friendly biological methods

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Abstract

Toxic mycobiota create ochratoxins, a class of mycotoxins, as secondary metabolites. All ochratoxins (A, B, and C) are structurally composed of polyketide dihydrocoumarin moiety derivatives connected to L-b-phenylalanine via an amide bond via the 7-carbon group. Among these, ochratoxin A (OTA) poses a serious hazard to public health globally and is frequently discovered in food and agricultural products to contaminate them. OTA's severe nephrotoxicity, immunotoxicity, neurotoxicity, and teratogenic mutagenesis need the development of efficient, affordable, and ecologically friendly decontamination and detoxification techniques. Maize is widely consumed crop and approximately 4.5 million tons of maize produces in Pakistan. 20 samples of maize from the Lahore market were collected, in which the ochratoxin content was determined by ELISA and positive sample were detoxified by biological method. Out of 20 maize samples 60% positive OTA (12/20) were found ranging from 3.5±0.08 to 40±1.67 ppb. 42% (5/12) of positive samples had the level higher than 5 ppb (as the EU standard allowed). The highly positive sample was detoxified by neem, moringa and olive leaf powder extracts (2.5, 5 and 10%)and result revealed that all extracts detoxify the ochratoxin-A up to 93.67±1.25 %, 88.15±1.12 and 82.79±1.05%, respectively at 10% concentration. In the present study significant variation in OTA content was observed in the maize samples and these extracts are excellent for detoxification of ochratoxin. Further assessments in other similar products and improvements were recommended in order to reduce the level of ochratoxin.

Keywords: Maize; Ochratoxin; ELISA; Detoxification; Olive; Moringa; Neem; Leave extracts

Introduction

When consumed, inhaled, or absorbed through the skin, mycotoxins secondary metabolites generated by filamentous fungus like Aspergillus, *Penicillium, Fusarium,* etc. can have both immediate and long-term negative effects on human and animal health. Everybody's health is greatly impacted by the worldwide presence of mycotoxins in food and feed, which also causes annual economic losses of billions of dollars (Santos *et al.* 2025). The most common mycotoxins found in feed include aflatoxin, ochratoxins, fumonisins, patulin, zearalenone, and trichothecenes, which include T-2 toxin and deoxynivalenol. Following aflatoxins, ochratoxin (A, B, and C) frequently contaminate agricultural items including corn, barley, oats, rice, wheat beans, coffee, and groundnuts. They also seep into animal products like eggs, milk, and meat including human milk. Secondary metabolites called mycotoxins is created by filamentous fungi. Ochratoxin A (OTA), the most deadly and prevalent member of the ochratoxin family, is one of several extremely toxic mycotoxins. OTA's nephro-toxic, genotoxic, cytotoxic, teratogenic, mutagenic, and immuno-toxic properties make it one of the top five mycotoxins that are strictly regulated by the law (Mwabulili *et al.* 2023).

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Climate affects its production: *Penicillium* species are more prevalent in cold climates, whilst *Aspergillus* species flourish in warm, temperate climates. For several Aspergillus species, the ideal temperature range for OTA production is between 25 and 30°C. *Penicillium verrucosum* is common in colder climates, but *Aspergillus ochraceus* is a major OTA generator in warmer places (Banahene *et al.* 2025). Renal cancers were found to arise in humans who consumed more over 70 µg/kg of OTA daily (Awuchi *et al.* 2022).

The IARC has designated ochratoxin A as a Group 2B probable human carcinogen. It is a powerful mycotoxin that is mainly recognized for its nephrotoxicity. Beyond the kidneys, it has teratogenic, mutagenic, hepatotoxic, and immune-suppressive effects (Figure 1). Low water solubility and high protein-binding affinity, with almost 99.98% binding to plasma proteins, primarily albumin, are characteristics of the OTA toxicokinetics profile that contribute to its lengthy half-life of 35 days (Khan *et al.* 2024). The OTA uses a number of methods to cause toxicity, such as disruption of mitochondrial respiration, suppression of protein synthesis, damage to DNA, and creation of oxidative stress. Although the exact process by which plant extracts modify OTA is unknown, alkaloids are thought to be involved (Jafarzadeh *et al.* 2024).

It is essential to concentrate on control measures to lessen OTA contamination in food. The significance of reducing mycotoxins for human and animal health has just been acknowledged in the last 60 years, since aflatoxins were discovered (Ding *et al.* 2023). Since pre- and post-harvest techniques using chemical or physical removal are insufficiently efficient, biological detoxification approaches are thought to be the most promising but difficult way to reduce the buildup of mycotoxins (Shahdeo *et al.* 2022).

In Pakistan, maize, rice and wheat are cultivated and consumed in large scales. These are considered as one of the primary energy sources in the world, being regarded as one of the highest yielding crops. Grown primarily for grain and fodder, maize is one of the most significant cereals produced worldwide for human and animal consumption. Over 80% of maize grain is utilized as feed, with the remaining portion being used to make semolina and starch.

Agriculture is the primary source of income in the majority of nations, including Pakistan. Food is contaminated by toxic fungi known as mycotoxin during storage, shipping, harvesting, growth, and processing. One-fourth of the food crops used for both human consumption and animal feed, such as maize, rice, wheat, barley, etc., are affected by mycotoxin worldwide. Mycotoxin has been related to numerous chronic illnesses, including cancer, blood and nerve abnormalities, and immunological suppression. Therefore, any mycotoxin,

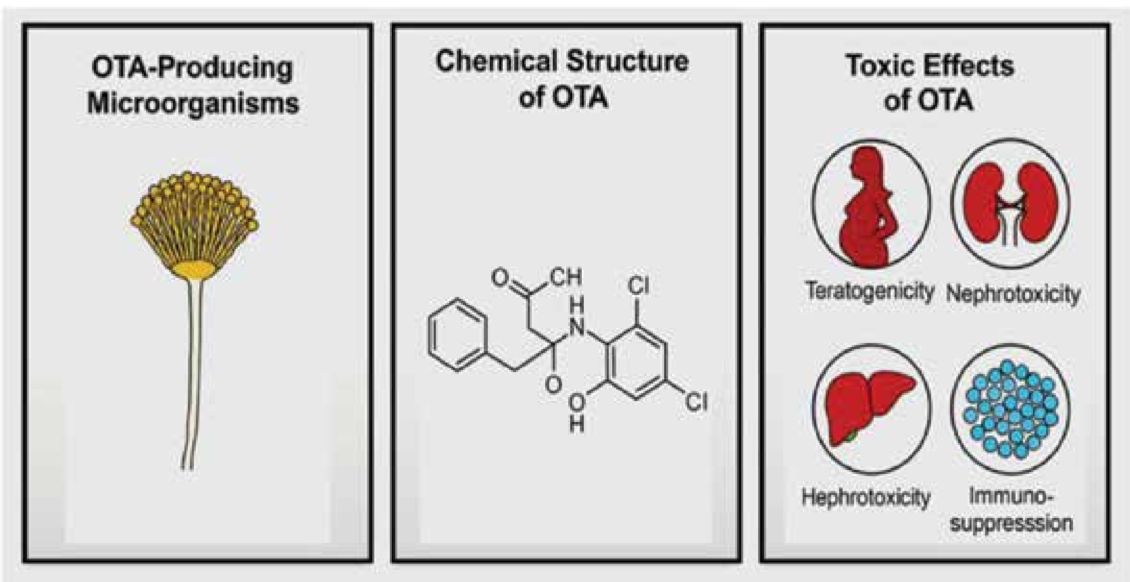

Fig. 1. Ochratoxin-A toxicity (Source: Khan *et al.* 2025)

such as ochratoxin-A, which can cause illness and financial losses if present in maize, must be removed from the diet and feed (Eskola *et al.* 2018). The main non-tariff trade barrier for agricultural products is mycotoxin contamination, which has a detrimental effect on small-holder farmers' health and income, regional and international trade, and the global economy. Strategies to lower the synthesis and prevalence of these mycotoxins in maize, as well as their penetration into the food and feed chains have been developed at both the pre-harvest and post-harvest crop phases (Palumbo *et al.* 2020). Ochratoxin knowledge in maize and its detoxification is crucial for maintaining food safety and public health. Strong carcinogens with a high cancer risk include ochratoxin-A. Therefore, it is crucial to employ effective methods to eliminate or reduce the amount of ochratoxin-A in maize. Keeping the view the present study the ochratoxin A was determined in various maize samples and positive samples were detoxified by various plant extract eco-friendly biological methods.

Materials and methods

Sampling and extraction

During the months of January-March 2026, the total of twenty (n=20) maize samples were purchased from local markets Lahore, Pakistan. Uniformly sized sample was prepared by grinding with warring crusher, stored in a plastic container in a refrigerator (4°C) and analyzed.

Ochratoxin A was extracted as described by Majeed (2017), briefly, a sample of 100 g was ground using the grinder to acquire a uniform size of 25 mm-mesh sieves and then stored at 4°C for analysis.

Quantitative analysis by ELISA

BIO-RAD (Modal 680, Germany) ELISA instrument was used to measure the levels of ochratoxin A in maize samples using the commercial kit (Neogen 8632 Veratox® HS ochratoxin) and a competitive inhibition enzyme immunoassay approach that followed the instructions given by the supplier. The limit of detection according to the ELISA kit technique is 2.0 ppb. Samples of maize were first processed by weighing 10g from each grinded sample. Each sample was shaken and mix well for 15 minutes with an addition of 40 ml of 70% methanol shake on a wrist action shaker for 30 minutes. After adding 100 µl of sample and 100 µl of conjugate enzyme to the wells, they were left in the dark at room temperature for 30 minutes. Following the washing procedure, substrate was added and after 15 minutes of incubation under the same conditions, absorbance at 650 nm was measured (Gumus *et al.* 2018).

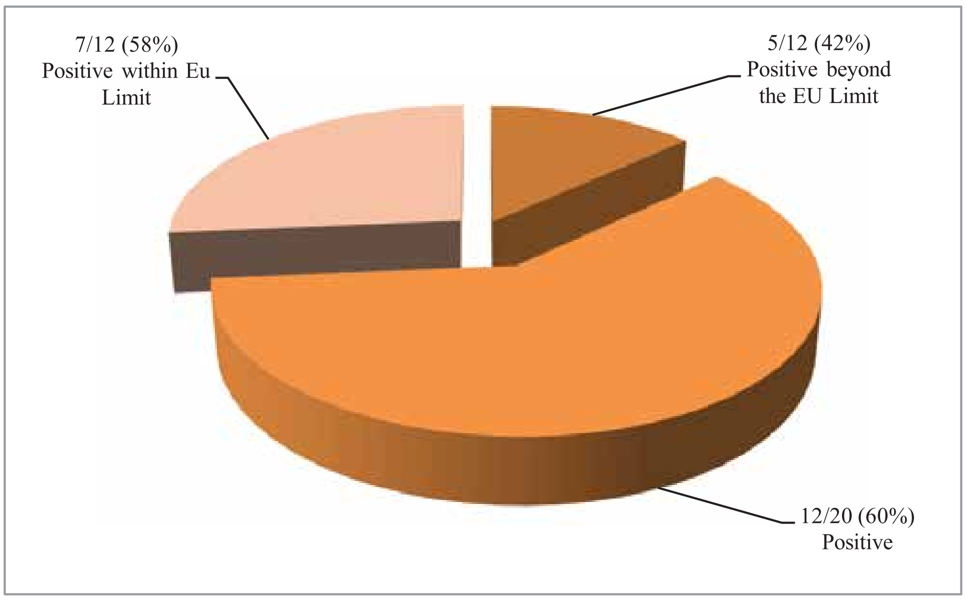

Fig. 2. Percentage of contaminated milk samples by ELISA

Biological detoxification of Ochratoxin A

Treatment with neem, olive and moringa leaves were bought was prepared by drying its leaf after washing and grinding. 100 ml of water was added to 2.5, 5.0 and 10 g of neem, moringa and olive leaf powder and well mixed by shaking. A 10g contaminated sample of maize was immersed in these solutions all day. The samples were filtered, cleaned and examined to see if the amount of ochratoxins has decreased.

Statistical analysis

SPSS software (SPSS 16.0 for Windows; SPSS Inc., Chicago, IL, USA) was used to examine the variance (ANOVA) of all the data. Duncan's multiple range test was used to determine significant differences between groups at $p<0.05$.

Results and discussion

Ochratoxin-A was quantitatively examined utilizing the ELISA Technique. Out of 20 maize samples 60% positive OTA (12/20) were found ranging from 3.5±0.08 to 40 ±1.67 ppb while other (8/20) 40% samples have no ochratoxin. Out of positive samples 58% (7/12) had the level higher than 5ppb (as the EU standard allowed) while other (5/12) samples have ochratoxin within the limit as shown in figure2.

Food crops are inevitably affected with mycotoxins due to a variety of reasons, including agricultural practices, geographic location, agronomic methods, storage times, etc. If maize, rice, and wheat are not properly stored after harvest, they may get contaminated. There are numerous ways to lessen mycotoxin contamination, including chemical, physical, natural, and biological approaches (Marrez *et al.* 2018). Reducing mycotoxins in crops requires safe or biological techniques. Ochratoxin-A in maize can be effectively reduced by using medicinal plant extracts such as neem, moringa, and olive leaf powder. As far as we are aware, no one uses these plant extracts to detoxify maize of ochratoxin. Additionally, using these plant extracts may guarantee that the breakdown process preserves the nutritional value rather than introducing new toxic or carcinogenic-mutagenic substances. It may also eliminate Aspergillus spores and mycelia, preventing the growth and production of new toxins under favorable conditions (Awuchi *et al.* 2021).

The highly positive sample was detoxified by neem, moringa and olive leaf powder extracts (at concentration 2.5, 5 and 10%) and result revealed that all extracts detoxify the ochratoxin-A ranging from 67.41±0.70–93.67±1.25% the difference between samples were $p<0.05$. Maximum ochratoxin-A reduction was observed (93.67±1.25%) in maize sample by using neem leaves extract (10%). The moringa extract reduced ochratoxin-A in maize (88.15±1.12%) while olive leaves extract reduce ochratoxin-A up to 82.79±1.05% by using 10% extract. The sequence of reduction level are neem extract> moringa>olive leaf extract (Table 1).

Among the most impacted crops are cereals, such as rice, corn, wheat, and oats, which cause large financial losses (Liu *et al.* 2022). Because it frequently occurs in nature and produces clinical disorders, ochratoxin A is an extremely significant toxin. Cereal grains, fresh and dried fruits, animal feed, beef and meat products, and fresh cheese have all been shown to contain OTA, which spoils about 10% of the world's agricultural products and results in losses of billions of euros (Abraham *et al.* 2025). Notably, 50% of agricultural products contaminated with OTA come from cereals alone, despite the fact that OTA is widely present in a variety of food and feed sources (EFSA, 2023). OTA contamination is, however, spreading more extensively than previously believed, according to new findings. For instance, OTA has been detected in 85% of Tunisian wine samples (Ahmad *et al.* 2024) and 91.5% of Nigerian cocoa beans (Banahene *et al.* 2024). OTA was found in 3,657 out of 6,857 cereal-based products in Canada, with a maximum content of 631 μg/kg (Walkowiak *et al.* 2022). Effective solutions, including as interventions during food processing and preventive measures during production and storage, are necessary to mitigate OTA contamination. The possibility to lower OTA levels has been investigated using chemical techniques like adsorbents and ozone (O_3), as well as physical techniques like gamma ray or electron beam irradiation (Khalil *et al.* 2021; Gonzalez *et al.* 2020). In a similar vein, biological techniques that employ extracts, enzymes, bacteria, and yeasts have potential due to their efficiency, specificity, and low influence on food quality (Shukla *et al.* 2020).

The findings of this study are constant with those of Zahra *et al.* (2020), who detoxified the ochratoxin-A in corn using black seed oil and adage a reduction level of 49.49%. According to Xiong *et al.* (2021), biological approaches are highly effective and selective for

Table 1. Detoxification of ochratoxin-A in maize sample by biological methods

Sl. No.	Method used for detoxification	Initial levels (ppb)	Levels after detoxification (ppb)	Reduction (%)
1	Neem leaf powder extract (2.5 %)	40.50±0.80	9.78±0.10	75.85±0.95
	Neem leaf powder extract (5%)	40.50±0.80	5.29±0.3	86.93±1.15
	Neem leaf powder extract (10%)	40.50±0.80	2.56±0.03	93.67±1.25
2	Moringa leaf powder extract (2.5%)	40.50±0.80	11.90±0.2	70.62±0.76
	Moringa leaf powder extract (5%)	40.50±0.80	8.30±0.5	79.50±0.83
	Moringa leaf powder extract (10%)	40.50±0.80	4.80±0.4	88.15±1.12
3	Olive leaf powder extract (2.5%)	40.50±0.80	13.20±0.3	67.41±0.70
	Olive leaf powder extract (5%)	40.50±0.80	10.18±0.10	74.86±0.94
	Olive leaf powder extract (10%)	40.50±0.80	6.97±0.16	82.79±1.05

Data are represented ± SD

detoxifying ochratoxin-A while not reducing the nutritional content of food. In this competition, Naeem *et al.* (2023) detoxify the ochratoxin-A by using *Amaran thusviridis* and *Sorghum helepense* and obtained promising results. They also manage the ochratoxigenic fungi by using *Sorghum helepense* (L.) extract and found 84% inhibition in its 2% methanol leaf extract against *C. cladosporoides* (Naeem *et al.* 2021). These plant extracts are being used for the first time to detoxify ochratoxin-A in maize. They contain phytochemicals such as polyphenols, alkaloids, flavonoids (quercetin, kaempferol, apiginin), and rutin. Flavonoids have a biochemical impact that inhibits the interest of enzymes and hormone regulation and feature antimicrobial, antiviral, anticancer, and antioxidant activities (Saeed *et al.* 2025). Therefore, the utilization of these plant extracts in the current study was highly promising in controlling the fungus that produce ochratoxin.

A basic human right is the availability of wholesome food free from unwanted contamination. Certain mycotoxins, particularly ochratoxin-A (OTA) pollution in food and feed, can lead to teratogenic issues, acute liver injury, inflammation, and liver cancer. Biological methods have been taken into consideration among the physical, chemical, and biological methods used to prevent the production, reduction, elimination, and deactivation of OTA in contaminated food because of their maximum efficiency, low cost, eco-friendliness, and non-degradation of nutritional quality. The use of powdered plant leaves (such as neem, moringa and olive) or their extract is very promising to break down these toxic compounds into non-toxic metabolites. It prevents nutritional loss in food and feed containing maize, providing a safer, more environmentally friendly option than chemical or physical approaches. Due to their low cost and high value (antioxidant rich extracts), these are particularly practical and efficient for detoxifying ochratoxin-A on a commercial and industrial level.

Conclusion

In this study the maize samples analyzed, it was found that the quantities of ochratoxin-Ain 60% were positive and 25% higher the allowed limit for maize. The highly positive sample was detoxified by neem, moringa and olive leaf powder extracts (at concentration 2.5, 5 and 10%) and result revealed that all extracts detoxify the ochratoxin-A ranging from 67.41±0.70–93.67±1.25%. The highest OTA reduction rates (93.67±1.25%) in maize were found by utilization of neem leaf extract. However, in order to preserve maize quality and safety in the face of rising global demand, ongoing monitoring and evidence-based practices are essential.

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