Aaatoxins 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. Aatoxin 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 aatoxin 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 aatoxins in various spices and contaminated sample were detoxified by black seed oil.

Results:
From this study aatoxins were detected in 70% and 30% spices samples have no aatoxin. Among contaminated samples 43% had aatoxins beyond the permissible limits whereas 57% had the aatoxins below the permissible limits. The positive samples were alleviated by biological method i.e. black seed oil (1-10%) which detoxified aatoxin in fennel 49.52 ± 1.50 – 92.50 ± 1.94% and detoxication 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 aatoxin levels in some spices.

Some types of mould in grains, nuts, spices, and dried fruits contain mycotoxins, which are harmful fungal 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...
sativa, also known as black seed or kalonji, is a member of

*Corollaria* family and is considered lucky to have
detrimental impacts on humans is rising [16, 17]. This is
main objectives of this study was to determine the

inactivate or reduce their bioavailability in contaminated

Atoxin extraction from spices was done by using the

Total. Aatoxin detection can be achieved by physical

UV light, heat, or ionizing radiation), chemical (adding

Thick Layer Chromatography (TLC)

Using a micro syringe, samples 5, 10, 15, 20, and 25μl were

samples 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.

**Atoxins Extraction and Analysis**

Atoxin extraction from spices was done by using the

Atoxins Extraction and Analysis

Atoxin 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.

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

Where:

S = Atoxin standard volume in μl

W = Weight of sample

V = Volume of extract

Y = Conversion factor (μg/μl)

Z = Volume of TLC plate

The following formula was used to calculate the aflatoxin

concentration:

**Calculations:**

The following formula was used to calculate the aflatoxin

concentration:
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
°C. 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

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Samples Names</th>
<th>Aflatoxins (ppb)</th>
<th>Permissible (EU limits)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Red pepper</td>
<td>40.82 ± 1.38</td>
<td>ND ND ND 50 ppb</td>
<td>Fit</td>
</tr>
<tr>
<td>2</td>
<td>Turmeric</td>
<td>6.27 ± 0.08</td>
<td>ND ND ND 10 ppb</td>
<td>Fit</td>
</tr>
<tr>
<td>3</td>
<td>Clove</td>
<td>ND ND ND 10 ppb</td>
<td>ND ND ND</td>
<td>Fit</td>
</tr>
<tr>
<td>4</td>
<td>Fennel</td>
<td>70.50 ± 1.6</td>
<td>ND ND ND 10 ppb</td>
<td>Fit</td>
</tr>
<tr>
<td>5</td>
<td>Cumin Seed</td>
<td>9.86 ± 0.12</td>
<td>ND ND ND 10 ppb</td>
<td>Unfit</td>
</tr>
<tr>
<td>6</td>
<td>Fenugreek</td>
<td>20.42 ± 0.42</td>
<td>ND ND ND 10 ppb</td>
<td>Unfit</td>
</tr>
<tr>
<td>7</td>
<td>Black Pepper</td>
<td>ND ND ND 10 ppb</td>
<td>ND ND ND</td>
<td>Fit</td>
</tr>
<tr>
<td>8</td>
<td>Cinnamon</td>
<td>ND ND ND 10 ppb</td>
<td>ND ND ND</td>
<td>Fit</td>
</tr>
<tr>
<td>9</td>
<td>Garlic</td>
<td>2.17 ± 0.06</td>
<td>ND ND ND 10 ppb</td>
<td>Fit</td>
</tr>
<tr>
<td>10</td>
<td>Ginger</td>
<td>8.88 ± 0.10</td>
<td>ND ND ND 10 ppb</td>
<td>Fit</td>
</tr>
</tbody>
</table>

*ND means Not Detected

In this study aflatoxin contaminated samples were
detoxified 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 Aatoxins in Fennel Sample by Black Seed Oil

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

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 Atoxins in Cumin Seed by Black Seed Oil

<table>
<thead>
<tr>
<th>Concentration of Black Seed Oil for Detoxification of AF</th>
<th>Initial Levels (ppb)</th>
<th>Levels after Detoxification (ppb)</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Seed Oil (1%)</td>
<td>9.86 ± 0.12</td>
<td>4.40 ± 0.086</td>
<td>55.37 ± 1.52</td>
</tr>
<tr>
<td>Black Seed Oil (2%)</td>
<td>9.86 ± 0.12</td>
<td>3.82 ± 0.06</td>
<td>61.25 ± 1.56</td>
</tr>
<tr>
<td>Black Seed Oil (3%)</td>
<td>9.86 ± 0.12</td>
<td>3.04 ± 0.04</td>
<td>69.17 ± 1.60</td>
</tr>
<tr>
<td>Black Seed Oil (5%)</td>
<td>9.86 ± 0.12</td>
<td>2.18 ± 0.02</td>
<td>77.89 ± 1.75</td>
</tr>
<tr>
<td>Black Seed Oil (10%)</td>
<td>9.86 ± 0.12</td>
<td>1.25 ± 0.01</td>
<td>87.32 ± 1.83</td>
</tr>
</tbody>
</table>

**Table 4:** Detoxification of Aatoxins in Fenugreek by Black Seed Oil

<table>
<thead>
<tr>
<th>Concentration of Black Seed Oil for Detoxification of AF</th>
<th>Initial Levels (ppb)</th>
<th>Levels after Detoxification (ppb)</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Seed Oil (1%)</td>
<td>20.42 ± 0.42</td>
<td>10.15 ± 1.32</td>
<td>50.20 ± 1.50</td>
</tr>
<tr>
<td>Black Seed Oil (2%)</td>
<td>20.42 ± 0.42</td>
<td>8.50 ± 1.02</td>
<td>58.37 ± 1.53</td>
</tr>
<tr>
<td>Black Seed Oil (3%)</td>
<td>20.42 ± 0.42</td>
<td>6.75 ± 0.40</td>
<td>66.94 ± 1.58</td>
</tr>
<tr>
<td>Black Seed Oil (5%)</td>
<td>20.42 ± 0.42</td>
<td>5.02 ± 0.08</td>
<td>75.41 ± 1.67</td>
</tr>
<tr>
<td>Black Seed Oil (10%)</td>
<td>20.42 ± 0.42</td>
<td>3.60 ± 0.06</td>
<td>82.37 ± 1.75</td>
</tr>
</tbody>
</table>

**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
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, aflatosins 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 organoleptic 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 as 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].

C O N C L U S I O N S
This study reveals that the biological method using 10% black seed oil worked for detoxification of aflatoxin in spices.

A u t h o r s  C o n t r i b u t i o n
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.

C o n f l i c t s  o f  I n t e r e s t
The authors declare no conflict of interest.

S o u r c e  o f  F u n d i n g
The authors received no financial support for the research, authorship and/or publication of this article.

R E F E R E N C E S

Saeed MK et al., Using Black Seed Oil for Detoxification of Aflatoxins
DOI: https://doi.org/10.54393/df.v5i2.134


