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EVALUATE THE PREVALENCE OF FUNGI AND MYCOTOXINS IN CORN STORED AT THE SILOS IN BAIJI CITY

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Abstract: Objective: The purpose of this study was to identify the fungus species found in corn and the mycotoxins they generate in different locations of grain storage and silos in Baiji City, Iraq.

Method: multiple one-kilogram weight samples have been obtained from different storage facilities and silos in Baiji city, each containing corn. Potato Dextrose Agar was employed to isolate and purify the fungi in corn. The fungi were identified using the polymerase chain reaction technique and by examining their physical and microscopic properties. The detection of mycotoxins in terms of quantity and quality was performed using the HPLC approach.

Results: The Study exhibited the presence of multiple fungal genera associated with corn in the storage and silos of Baiji city. Such as Aspergillus flavus, Aspergillus Niger, penicillium viridicatum, Alternaria alternata, and Fusarium solani. Aspergillus had the highest occurrence and frequency percentage. In contrast, Alternaria had the lowest frequency percentage, and Penicillium had the lowest occurrence percentage. LIKEWISE, each fungal strain produced a variable of mycotoxins. Aspergillus flavus produced 214.1 ppb of aflatoxin B1, penicillium viridicatum 135.0 ppb of ochratoxin A, Alternaria alternate 905.1 ppb of alternariol, and Fusarium solani 1025 ppb of fumonisin B1.

Conclusion: The study outcomes exhibited that the maze stored in the examined areas showed contamination by different Mycotoxigenic Fungi, such as Aspergillus flavus, Aspergillus Niger, penicillium viridicatum, Alternaria alternata, and Fusarium solani. These outcomes can be attributed to environmental factors during storage, such as temperature and moisture content, which impact fungi growth and mycotoxin production. Research data can assist in identifying strategies to produce unfavorable storage conditions that impede fungal growth and inhibit the release of mycotoxins.

Key words: storage corn, mycotoxins, aflatoxin B1, ochratoxin A, alternariol, fumonisin B1, corn silos, Baiji city.

Introduction:

Fungi are heterotrophic creatures that get nutrients via parasitism on live organisms or by living saprophytic, feeding on decomposition plant matter. Hence, fungal organisms can infect crops and lead to a drop in yield [1]; fungal contamination of food is widely Recognized as a substantial hazard to food safety and protection. Filamentous fungi can produce very dangerous secondary metabolites known as mycotoxins. This is the reason for it. Mycotoxins are the primary pollutants found in agricultural goods and account for a substantial portion of the projected yearly global food loss of 1.3 billion metric tons. Mycotoxins are present throughout the food chain due to fungal contamination during cultivation. Additionally, further infection can occur during the storage and processing of food. [2] The primary fungal genera capable of synthesizing

mycotoxins include Aspergillus, Fusarium, and Penicillium Aspergillus. flavus is the primary species of Aspergillus that infects various crops, including maize, peanuts, and spices. This species possesses the ability to produce aflatoxins. Aflatoxin is categorized as a significant peril to the health of both humans and animals because of the elevated levels of contamination found in food. **[3] ,** Spores of aflatoxin-producing fungi can naturally be found in certain food products. When the conditions are suitable, these fungi can create significant quantities of aflatoxins. The foods listed are maize, sorghum, pearl millet, rice, wheat, groundnuts, soybeans, sunflower seeds, cotton seedcake, chilies, coriander, turmeric, and ginger. [4] . Ochratoxin, produced by many species of Aspergillus and Penicillium, is one of the most prevalent mycotoxins that contaminate food. Additionally, water-damaged dwellings and heating ducts commonly contain this impurity. Polluted food products, particularly grain and pork, coffee, wine, and dried grapes, can expose humans to it. The toxin has been detected in the tissues and organs of various species, including human blood and breast milk. [5] .

Alternariol, a poisonous metabolite produced by Alternaria fungus, is a significant pollutant in grains and fruits. It possesses antifungal and phytotoxic properties. Researchers have observed that it hinders the activity of cholinesterase enzymes. [6][7], On the contrary, Fumonisin B1 is the predominant member of a group of poisons called fumonisins. Several species of Fusarium molds, particularly Fusarium verticillioides, create these toxins, commonly found in maize (corn), wheat, and other cereals. Global studies have documented maize contamination with Fumonisin B1, with levels measured in milligrams per kilogram. Human exposure to this substance ranges from micrograms to milligrams per day, and it is highest in areas where maize products are the central part of the diet.

The growth of fungi and the generation of mycotoxins are highly influenced by temperature and the moisture contents of the substrate. Temperature and moisture conditions are essential for fungal development, and mycotoxin generation alters depending on the particular fungal species, strains, and types of mycotoxins. The 20 to 30 °C temperature range is the most favorable for the greatest generation of Aspergillus toxins, including aflatoxins and ochratoxins. Fusarium toxins, including fumonisins and trichothecenes, are generated at temperatures varying from 15 to 30 °C.[8], Another study observed that Aspergillus toxins need an adequate water activity level of 0.9 aw. The production of most Fusarium toxins is most favorable with a water activity (aw) of 0.9 or higher. However, some can still be produced at a water activity of 0.86 or higher.

Multiple investigations were conducted to evaluate the contamination of fungal spores in silos and storage facilities in an Iraqi city. One of these studies identified Aspergillus flavus as the primary causal agent for the contamination of stored grains in multiple Iraqi cities. This particular fungus is known to create aflatoxin. The study found that temperature and moisture content are the primary environmental elements that influence the presence of fungus and the development of aflatoxin. Additionally, another study discovered that aflatoxin B1 contamination in maize harvests stored in silos in Baghdad was more prevalent compared to other crops.[10][9]

However, these investigations have failed to investigate the extent of fungal contamination and its mycotoxins levels in corn storage and silos at baiji city.[11][12] Our study aimed to examine the diverse forms of fungi and mycotoxins linked with the storage of corn in silos and storage facilities in Baiji City, Iraq.

Material and method:

1- Samples Collection:

We gathered a diverse assortment of 1-kilogram maize samples from grain silos and stored them in Baiji City, Iraq. The process of collecting specimens commenced on January 1, 2024. We stored the specimens

in polyethylene bags, marking them with the corresponding sample number and the collection date. After that, we tightly sealed the samples and refrigerated them at five °C until their use.

2- Evaluation of Corn Seed Health:

2-a - Isolation and purification of fungi:

The fungi associated with the corn grains were isolated using the agar plate method. We cultured the corn samples in potato dextrose agar (PDA) and potassium dextrose agar media containing streptomycin (100 ppm). Before being dispersed onto the dishes, the grain samples were sterilized with a 4% sodium hypochlorite (NaOCl) solution for two minutes. **[13]** Subsequently, the dishes were incubated at a precise temperature of 26°C for seven days; the fungi that were separated and kept separate from other organisms were cleansed and determined using a microscope and the categorization system described in the specified keys.[14] .

2-b- identification of Fungi from corns was made based on the following:

a- Morphological characteristic:

After isolating fungi, they are identified based on their morphological and microscopic characteristics. Their growth patterns and colony morphology determine fungi,[15] spores, organization, and composition. [16] **,**

b- Polymerase Chain Reaction:

We used the PCR technique to identify the fungus isolated from corn precisely. The fungal DNA extraction procedure entailed amplifying the internal transcribed spacer region via primers. Using PCR kits provided by Chelex®100, we effectively isolated DNA from a single pure and active fungal colony. The extraction process followed the directions provided by the company. [17]

The frequency and occurrence percentage of the isolated fungus was evaluated using the following equations after identification:

$$\boldsymbol{frequency}\ \% = \frac{number\ of\ isolated\ fungi}{total\ number\ of\ isolated\ fungi} * 100\% \qquad \textbf{Occurrence}\ \ \% = \frac{number\ of\ samples\ containing\ fungus}{total\ number\ of\ samples} * 100\%$$

3- Quantitative and qualitative Detection of mycotoxins by HPLC:

The Ministry of Science and Technology laboratories conducted the investigation, emphasizing quantitative and qualitative factors. The mycotoxins were detected using SYKAM HPLC equipment manufactured in Germany.

3-A- Identification of the alternariol mycotoxin:

Identification of alternariol involved: 25 g from the corn sample was weighed in a 100-ml centrifuge tube, and a 30 ml extraction mixture (CH3CN/H2O/MeOH, 45/45/10, v/v/v modified to pH 3 with add o-phosphoric acid). A polytron mixed the mixture at 10,000 rpm for 4 minutes; PH was adjusted to 3 using concentrated o-phosphoric acid. Following a 5-minute centrifugation at 4,000 rpm, 15 ml of the supernatant was diluted with phosphate buffer (0.05 M sodium dihydrogen phosphate adjusted to pH=3) to 50 ml, then shaken for 1 minute. Twenty milliliters of the diluted sample extract were passed through a conditioned SPE cartridge (conditioning of the SPE cartridges was made first with 5 ml MeOH, followed by 5 ml water). The cartridge was rinsed with 5 ml of water and then air-dried on the manifold. Then, Alternaria toxins were

eliminated sequentially with 5 ml MeOH and 5 ml CH3CN. After dryness by evaporating, the cleaned-up extract was dissolved in 500 µl of CH3CN (3/7,[18] v/v), including 0.1% formic acid, and then injected into the HPLC system.

Quantifying alternariol mycotoxin was done using the HPLC model SYKAM (Germany). The mobile phase was isocratic methanol: acetonitrile: D.W (15 mg ammonium hydrogen carbonate dissolved in 150 ml D.W) (10: 60: 30) (v/v) mixture with a flow rate of 1.0 mL/min. The column was C18 – ODS (25 cm * 4.6 mm), and the detector UV-Vis at = 280 nm).

3-B- Identification of the aflatoxin B 1 mycotoxin:

Identification of aflatoxin B1 involved: after homogenizing 10 grams of each item under examination for 90 seconds using 40 mL of acetonitrile, water (60:40, v/v), and 0.2 g NaCl, a magnetic stirrer was used to combine the mixture for 10 minutes. Fast filtering Whatman We used No. 1 filter paper (Whatman et al., USA) to filter the combination. In a 50-mL Erlenmeyer flask, 4 mL of filtrate was diluted with 44 mL of a 2% tween-20-PBS solution. After that, the filtrate was cleaned using the following liquid extraction method: We mixed 0.5 mL of acetonitrile with 0.5 mL of the filtrate aliquot, then pipetted 0.5 mL of the mixture into an Alltech. We packed a 1.5 mL Extract-Clean reservoir with 200 mg of basic aluminum oxide (9 mm high-layer adsorbent). Using 100 µL of the extract, the HPLC system (Sykam S 600 HPLC System Brochure, Germany) was used to detect the mycotoxins. [19].

Aflatoxin B-1 mycotoxin was quantified using the HPLC model SYKAM (Germany). The mobile phase was acetonitrile: D.W. (70: 30), a (v/v) mixture of the flow rate at 0.7 mL/min; the column was C18-ODS (25 cm * 4.6 mm), and the detector was UV-Vis at λ excitation wavelength = 365 nm and λ emission wavelength = 445.

3-C- Identification of the Ochratoxin A mycotoxin:

Ochratoxin was extracted from corn samples (30 g) by homogenization with 150 mL of acetonitrile: H2O (6:4, v=v) for 15 minutes. The extract was filtered, and 25 mL of the filtrate was diluted with 50 mL of phosphate-buffered saline, pH 7.4 (PBS). We degassed the samples in a sonic bath for 30 minutes. The pH was adjusted to 7.2 using two mM sodium hydroxide. 5 mL of acetonitrile was added to the sample and stored until the analysis was performed.

Quantification of ochratoxin A mycotoxin was produced by employing the HPLC model SYKAM (Germany). The mobile phase was isocratic acetonitrile. D.W.: formic acid (50: 47: 3) at a flow rate of 1.0 mL/min; the column was C18-ODS (25 cm * 4.6 mm); and the detector was Florescent (Ex = 360 nm, Em = 440 nm).

3-D- Identification of the Fumonisin B1 mycotoxin:

Identification of Fumonisin B1 involved homogenizing 10 g of sample in chloroform - methanol-water (1: 2 :1, v/v; 100 mL) for 3 min. After centrifugation, an aliquot of the supernatant was cleaned up using robust anion extraction (SAX), preconditioned with methanol and chloroform - methanol-water (1: 2 :1, v/v). After washing with methanol, the fumonisins were eluted with acetic acid-methanol (1:99, v/v; 10 mL), dried under nitrogen at 60 °C, and then stored at four °C before analysis.

We quantified the fumonisin B1 mycotoxin using the HPLC model SYKAM (Germany). The mobile phase was methanol: 0.1 m NaH2 PO4 (77:23; v/v), adjusted to pH 3.35 with o-phosphoric acid at a 1.0 mL/min flow rate. The column was C18-ODS (25 cm * 4.6 mm), and the detector was fluorescent (Ex = 335 nm, Em = 440 nm).

4- The physical parameters that influence the fungal infection of grain storage:

The physical variables include temperature, humidity, pH level, and several other constituents. The moisture was measured using a hygrometer placed at the grain storage locations, yielding various percentages. [20] .

5- Ethical approval:

There was no study about humans involved in the investigation.

6- Statistical analysis:

The data analysis for the study is conducted using the "Statistical Package for Social Science" (SPSS) program for Windows (Version 20). I was used to performing calculations for the Independent t-test and determine the p-value ($P \leq 0.05$). , occurrence, and Frequent percentages were calculated to determine which fungal isolate was the most prevalent.

Results:

Isolation and purification of fungi:

A range of corn-associated fungi were isolated and identified, including five species: Aspergillus flavus, Aspergillus nigra, Penicillium viridicatum, Alternaria alternata, and Fusarium solani. The percentages of these fungi were as follows: Aspergillus flavus (25%), Aspergillus Niger (33%), Penicillium viridicatum (6%), Alternaria alternata (18%), and Fusarium solani (27%). The occurrence percentages of the isolates were as follows: Aspergillus flavus (66.6%), Aspergillus Niger (13.4%), Penicillium viridicatum (6.6%), Alternaria alternata (40%), and Fusarium solani (17.9%). The frequency percentages were as follows: Aspergillus flavus (38.46%), Aspergillus Niger (30%), Penicillium viridicatum (9.24%), Alternaria alternata (6.15%), and Fusarium solani (26.15%). (as shown in (Table 1,2,3) (figure 1,2,3,).

However, the examination of fungal toxins revealed the presence of specific species. Aspergillus flavus was found to produce aflatoxin B1 at a concentration of 214.1 ppb—penicillium viridicatum Produced ochratoxin A at a concentration of 135.0 ppb; Alternaria alternata produced alternariol at 905.1 ppb; and Fusarium solani produced fumonisin B1 at 1025 ppb. (table 4) (figure 4,5,6,7,8).

The physical parameters that influence the fungal infection of grain storage:

The study results of the physical parameters of the storage and silos of baiji city revealed (a temperature of 34 C°, acidity of 5.9, and humidity of 17 %, while the salt was 1%. (Table 5).

Table (1): fungus species in Baiji City corn storage and silos

No	Fungal genus isolated	fungi presence in the silos of Baiji City	Percentage
1	Aspergillus flavus	+	25 %
2	Aspergillus Niger	+	33 %
3	penicillium viridicatum	+	6 %
4	Alternaria alternata	+	18 %
5	Fusarium solani	+	27 %
p-value			0.037
+: if the fungus is present, -: if it is not, * Significant at level (p<0.05),			

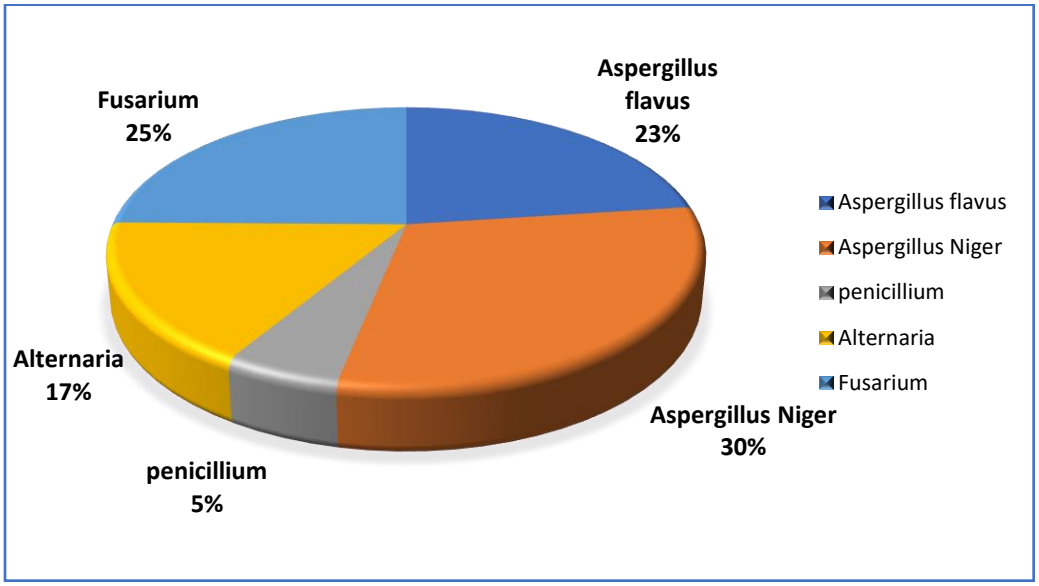

Figure (1): fungus species in Baiji City corn storage and silos

Table (2): occurrence percentage of the fungus species in Baiji City corn storage and silos

No	Fungal genus isolated	occurrence %
1	Aspergillus flavus	66.6 %
2	Aspergillus Niger	13.4 %
3	penicillium viridicatum	6.6 %
4	Alternaria alternata	40 %
5	Fusarium solani	17.9 %
p-value		0.012
* Significant at level (p<0.05),		

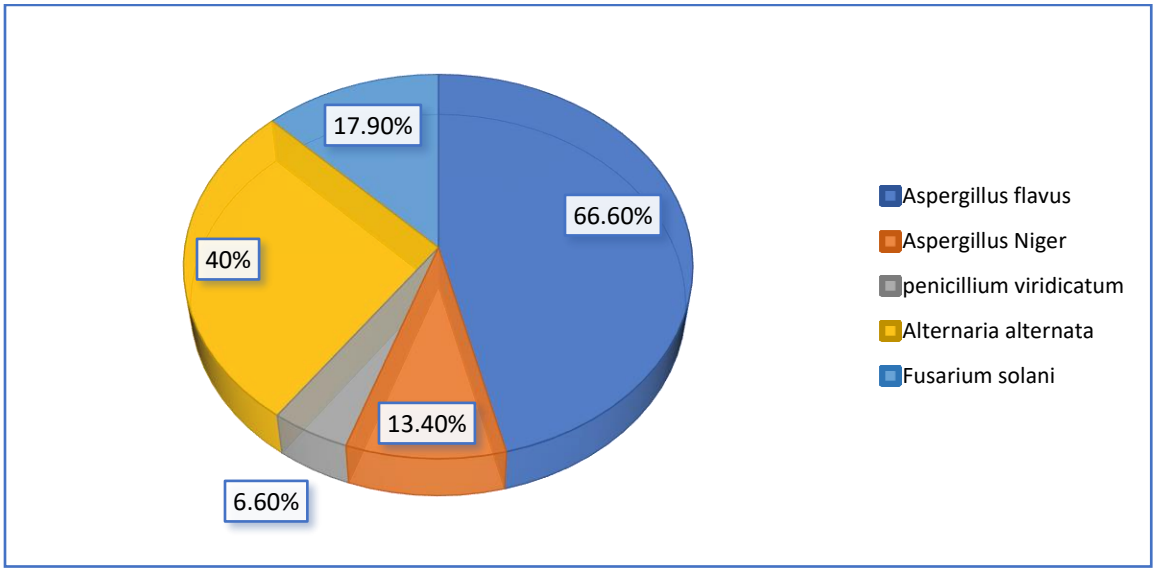

Figure (2): occurrence percentage of the fungus species in Baiji City corn storage and silos

Table (3): frequency percentage of the fungus species in Baiji City corn storage and silos

No	Fungal genus isolated	occurrence %
1	Aspergillus flavus	38.46 %
2	Aspergillus Niger	20 %
3	penicillium viridicatum	9.24 %
4	Alternaria alternata	6.15 %
	Fusarium solani	26.15 %
p-value		0.047
* Significant at level (p<0.05),		

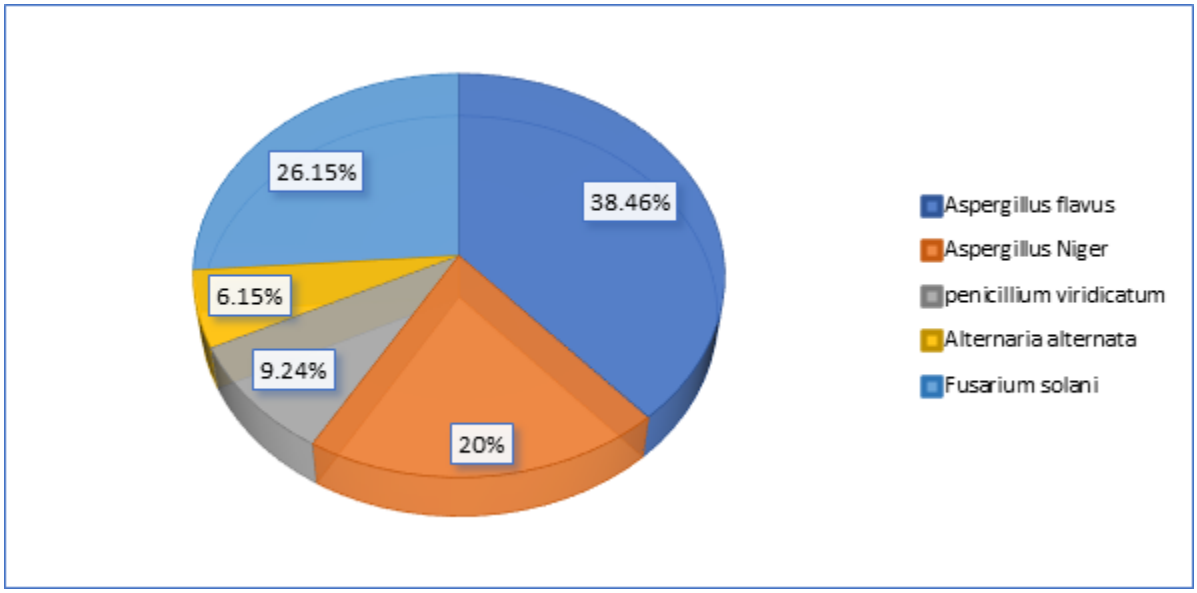

Figure (3): frequency percentage of the fungus species in Baiji City corn storage and silos

Table (4): Identification and quantification of mycotoxins generated by different isolated fungal species

No	Fungal genus isolated	Type of mycotoxin	Mycotoxin quantities / ppb
1	Aspergillus flavus	aflatoxin B 1	214.1
2	penicillium	Ochratoxin A	135.0
3	Alternaria alternata	alternariol	905.1
4	Fusarium	Fumonisin B1	1025
p-value			0.001
+: if the fungus is present, -: if it is not, * Significant at level (p<0.05),			

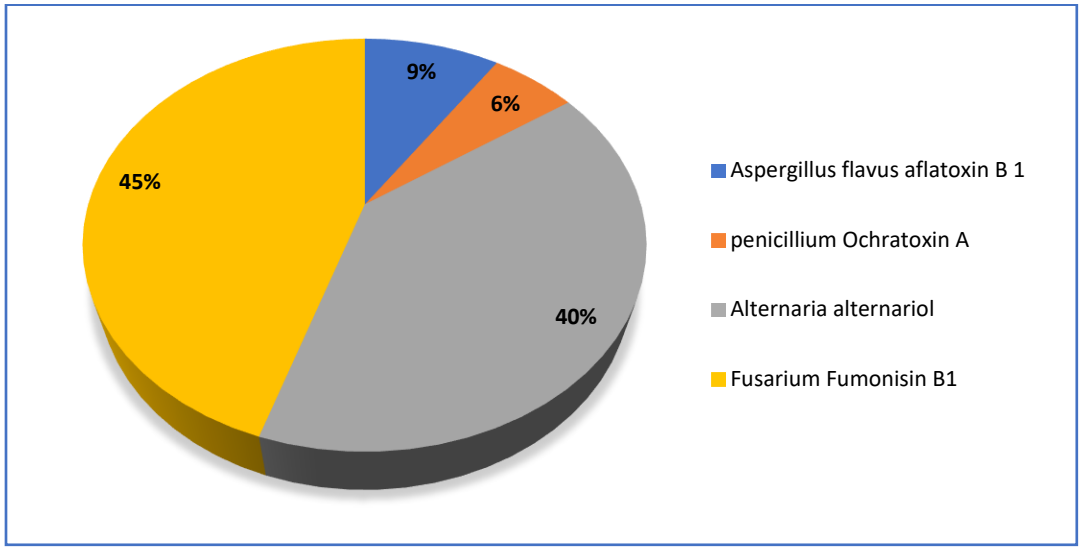

Figure (4): Identification and quantification of mycotoxins generated by different isolated fungal species

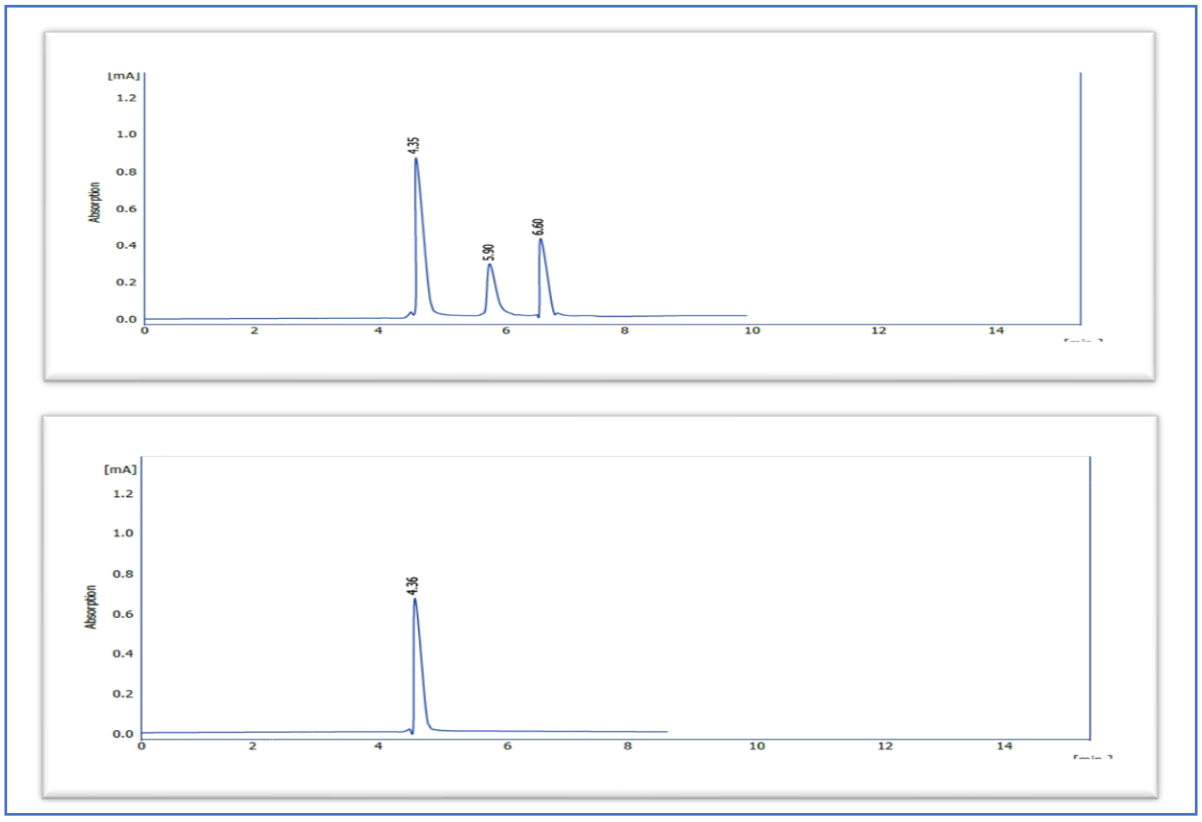

Figure (4): Chromatogram of aflatoxin B1 (standard)

Figure (5): The upper image displays the chromatogram of the sample, whereas the lower image shows the standard of aflatoxin B1.

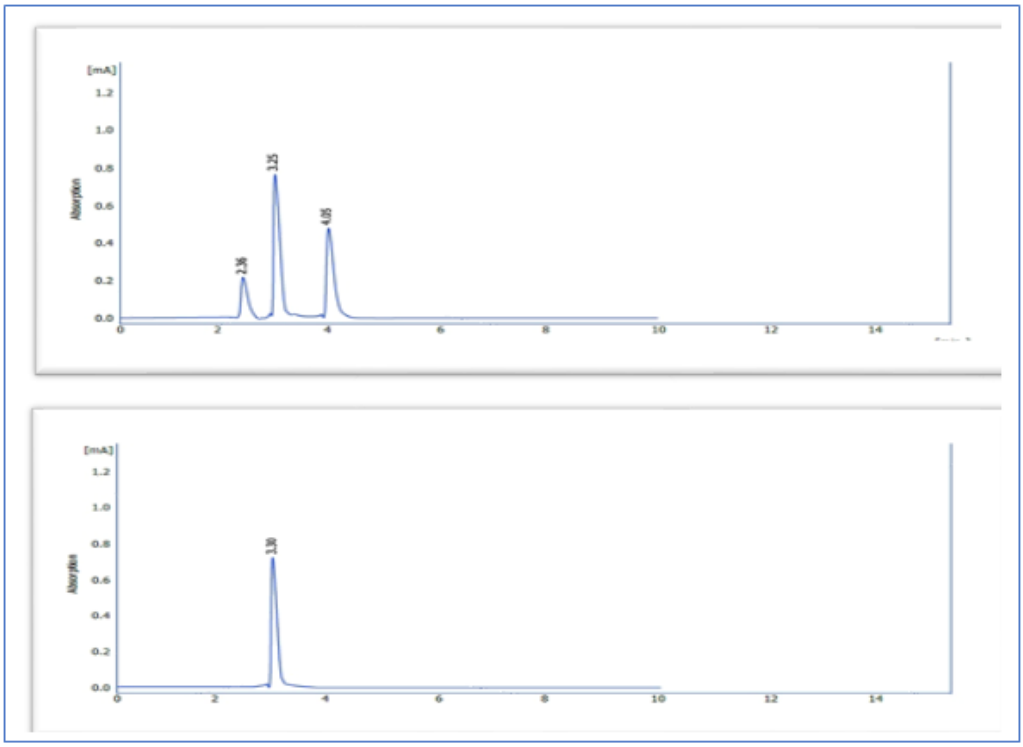

Figure (6): The upper image displays the chromatogram of the sample, whereas the lower image shows the standard of Ochratoxin A

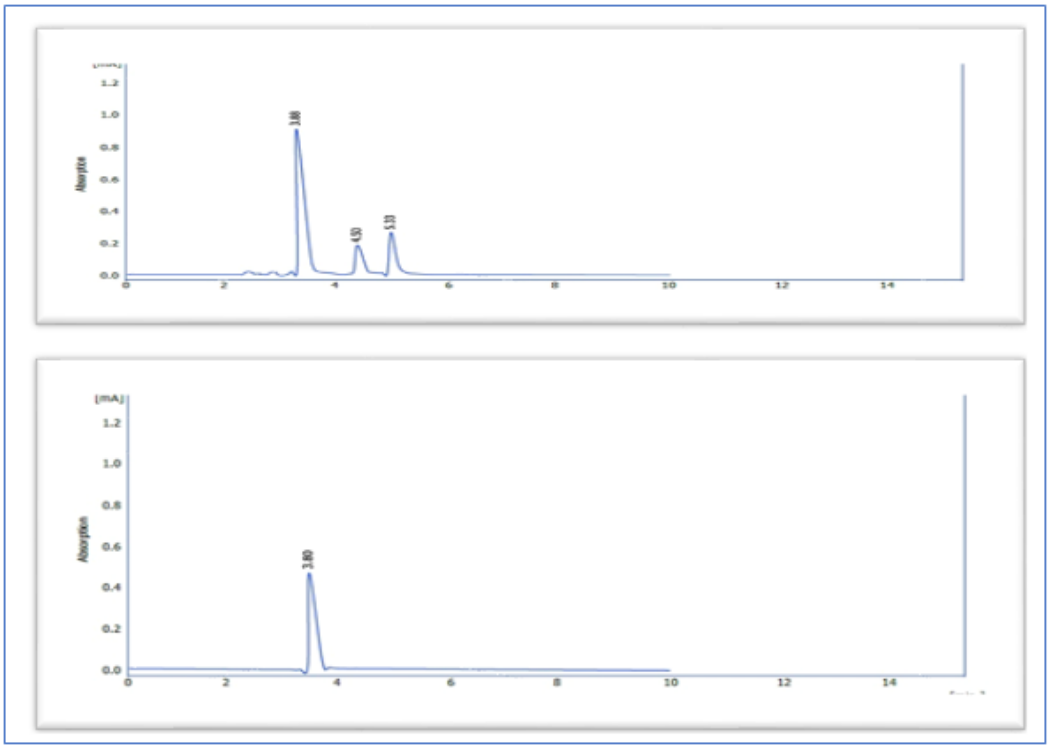

Figure (7): The upper image displays the chromatogram of the sample, whereas the lower image shows the standard of alternariol

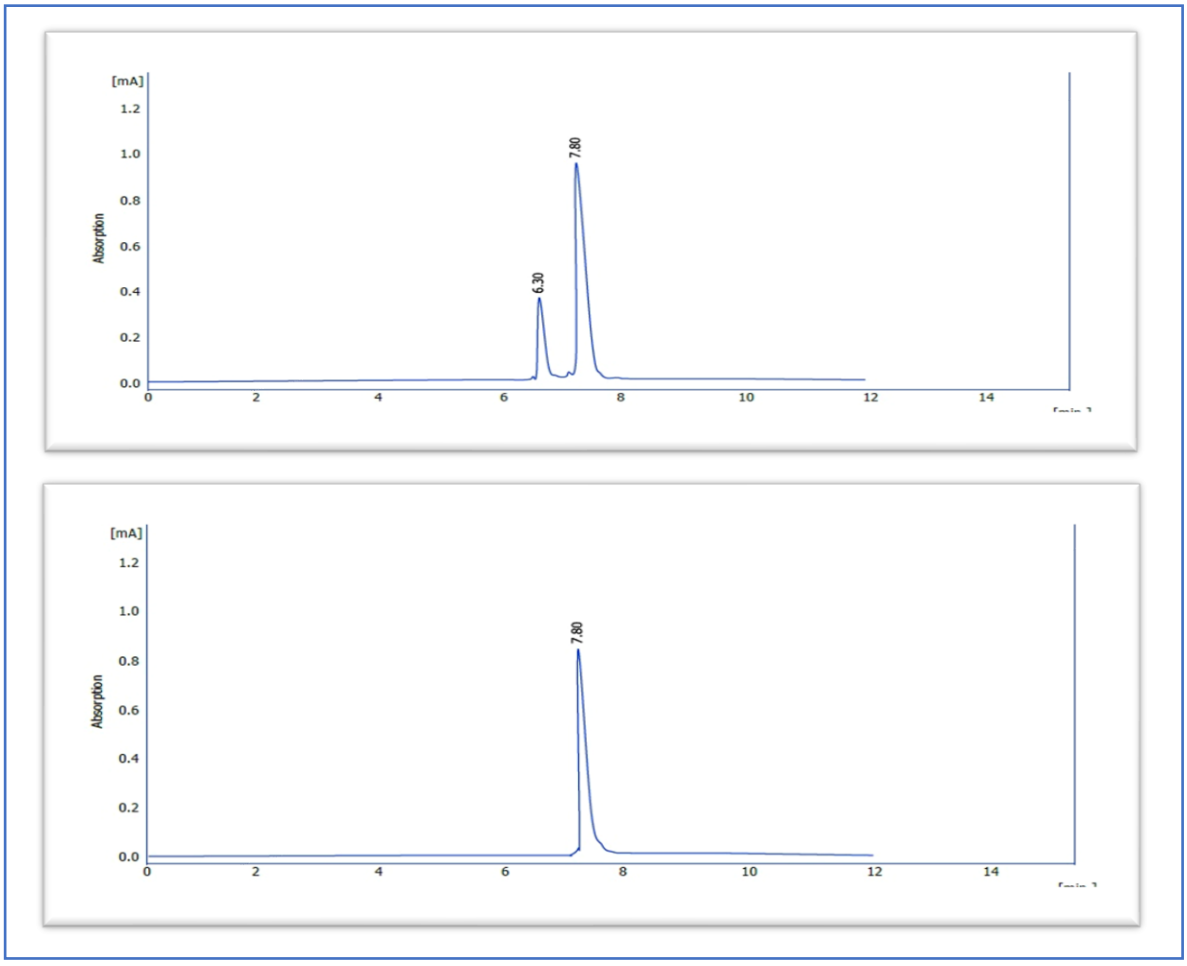

Figure (8): The upper image displays the chromatogram of the sample, whereas the lower image shows the standard of Fumonisin B1

Table (5): Mean values of physical characteristics in Bajie silos and storages

Physical parameters	Temperature	Acidity	humidity	salinity
Average reading	34	5.9	17%	1%

Discussion:

The outcomes of our study, which aimed to explore the fungal species associated with corn located in the silos of Baiji City, revealed the isolation of Aspergillus flavus and Aspergillus Niger. The occurrence and frequency of isolates demonstrated that Aspergillus spp had the highest isolates. Due to its high carbohydrate content, Aspergillus flavus is a natural contaminant of grains, especially corn. [21] . Moreover, specific vitamins and minerals [22] Facilitate the proliferation of A. flavus. Furthermore, it was shown that the spread of Aspergillus was mainly influenced by temperature. Specifically, temperatures above 20ºC were identified as a crucial factor**.**

In contrast to earlier studies, our analysis demonstrated the kinds of aspergillus. Flavus were aflatoxigenic, and water availability and temperature are the primary ecological factors influencing aflatoxin formation before and after harvest.

On the other side, the frequency percentage of Alternaria spp. Isolated from the silos of Baiji city was the lowest among the isolated fungus. This finding is consistent with another research that identified a correlation between Alternaria alternata and maize grains.[23] , A separate study found that out of 370 recently harvested wheat grain samples, 95% of the grains tested positive for several Alternaria toxins; this study presents the first documentation of Alternaria mycotoxins in Chinese wheat; in other studies, Alternaria species, notably A. alternata, heavily affected their wheat grains, with a median infection rate of 87.3%.

Penicillium spp. was also identified among the isolates. These findings are consistent with the results of three previously published research studies. **On the** other side**,** Fusarium spp. was also found in the silos of Baiji city, confirming previous research that identified corn contamination with Fusarium spp. [24]**.**

Our investigation determined that the isolated aspergillus was of the aflatoxigenic type. This finding aligns with another discovery that maize crops are susceptible to degradation by various fungi, including Fusarium, Penicillium, and Aspergillus. The strains of Aspergillus are toxigenic and produce B—and G-type aflatoxins.

Our study outcomes exhibited the presence of ochratoxin, produced by penicillium viridicatum, at a concentration equal to 135.0 ppb in stored corn. Study results align with those of others, who also detected contamination of corn and wheat samples with ochratoxin. Ochratoxin contamination was mainly seen in areas where moisture migration occurred due to insufficient ventilation and exposure to moisture from rainfall or condensation. The grain samples are characterized by significant ochratoxin contamination and a prevalence of penicillium spp.[25].

The study results also revealed the presence of Alternaria alternata, which exhibited the ability to produce alternariol at a concentration equal to 905.1 ppb. Other studies also showed this observation, which hypothesized that corn and wheat were contaminated with Alternaria toxins during the harvest seasons. Contamination was notably higher in wheat compared to corn. At the same time, the amount of toxins was higher in corn. In maize, the greatest concentration of total Alternaria toxins was 1,283 μg/kg. In contrast, the maximum value in wheat was 175.7 μg/kg. **[26]** . On the other hand, our investigation found that the level of Fumonisin B1 mycotoxin was 1025 ppb. Another study also showed similar results, indicating that contamination by fumonisin mycotoxins can occur in both symptomatic and asymptomatic grains. Higher temperatures in environments can potentially increase the generation of elevated fumonisin levels in corn.

Temperature is considered an essential factor in the domain of storage. It lowers the moisture content in grains and seeds to a level that protects them against fungal diseases during storage. Temperature is a significant environmental component that impacts aflatoxin formation by Aspergillus significantly. flavus, a prevalent fungus found in grain storage. The optimal development of this fungus occurs within the temperature range of 29-35°C. In this investigation, the silos and storage temperature were maintained at 34°C, which is considered the ideal temperature for the development of fungal mycelium.

Another study has demonstrated specific temperature ranges conducive to fungus development. The optimal temperature for the development of A. flavus is 36-38 °C, within the broader range of 8-46 °C. Moisture, oxygen levels, and nutrition availability influence the lowest and highest temperatures.

Elevating temperatures (27-45 °C) during growth and pollination of the grain reduce plant output and cause aflatoxins post-harvest. Nighttime temperature, nitrogen deficit, dense vegetation, irregular root growth, bird and insect wounds, and cracks affect aflatoxin production Humidity is a crucial factor in determining fungal

infection and aflatoxin production in silos and storage facilities. In our study, the humidity level in the silos and storages was measured at 17%. Notably, most fungi require a moisture content between 14% and 19%, which alters depending on the kind of grain being stored and the particular fungus in the storage area. Corn grains need less than 14-15% humidity to store. Otherwise, it would decay and blacken in days. High humidity accelerates contamination. Due to its high fat and low zoot content, maize grain stores less than other Gramineae cereals and secretes more aflatoxins.

The study was limited in its analysis of isolated fungi since it focused solely on a particular group of fungal taxa known to be detrimental to human and animal health due to their potential to create mycotoxins.

Conclusion:

The study identified five fungus genera that were present in maize storage in silos and the storage facilities in Baiji city. These genera include Aspergillus flavus, Aspergillus Niger, Penicillium, Alternaria, and Fusarium. Each fungal isolate in our study produced specific mycotoxins at varying levels. Aspergillus flavus produced aflatoxin B1 at a level of 214.1 ppb, penicillium viridicatum produced Ochratoxin A at a level of 135.0 ppb, Alternaria alternata produced alternariol at a level of 905.1 ppb, and Fusarium solani produced Fumonisin B1 at a level of 1025 ppb. These findings suggest that the storage conditions in the silos of Baiji City may have contributed to mycotoxin production. Our study analyzes mycotoxin contamination in corn grains stored in Baiji city, along with the associated storage conditions. This information can help identify necessary measures to establish unfavorable storage conditions that inhibit fungal growth and effectively prevent the release of mycotoxins.

Author Contributions:

Design and development:

Data collection and organization:

Statistical analysis and comprehension:

Composition of the article:

Reviewing the essay critically for key conceptual points:

Proficiency in statistical analysis:

Ultimate endorsement and guarantee of the article:

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Conflicts of interest :No conflicts of interest existed.

Abbreviation:

ppb: part per billion

spss: Statistical Package for Social Science

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