# CHAPTER: 14

# EVALUATION OF AFLATOXIN B1 CONTAMINATION IN CLUSTER BEAN (CYAMOPSIS TETRAGONOLOBA L.) SEEDS BY HPLC: FIELD AND STORAGE PERSPECTIVES

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## *ABSTRACT*

*Agricultural goods, such as cluster bean seeds, are greatly endangered due to the prevalence of moulds and mycotoxins. Using field and storage conditions as points of focus, this study follows the life cycle of moulds and mycotoxin contamination in cluster bean seeds. An in-depth investigation of the many elements impacting contamination risk is undertaken in order to provide light on efficient management approaches. To track the development of mould and mycotoxin levels, field surveys were carried out at various points in the cluster bean's life cycle. Furthermore, samples were taken both after harvest and throughout storage in order to determine how long the contamination lasted and whether it could have been amplified. To learn how different storage conditions, agricultural techniques, and environmental factors affected mould growth and mycotoxin generation, we conducted an evaluation. Moulds are common in cluster bean fields, according to the results, and the species composition varies by region and climate. It was also discovered that mycotoxin levels varied during the growth season, sometimes reaching dangerously high levels. Improper storage conditions amplified contamination risks, and post-harvest handling and storage techniques were also critical. In order to develop effective mitigation methods, it is crucial to understand the factors that contribute to the contamination of cluster bean seeds with mould and mycotoxins. To minimise contamination concerns, it is vital to implement integrated approaches that include sound farming practices, proper post-harvest management, and optimised storage conditions. To further stop the spread of infected seeds, it is advised to implement early detection methods and monitoring systems to pinpoint areas of contamination. In order to reduce the possibility of mould and mycotoxin contamination in cluster bean seeds, this study stresses the significance of taking a comprehensive approach that takes field and storage conditions into account. Seed quality, food safety, and public health can all be improved if stakeholders work to eliminate the main causes of contamination.*

***Keywords:*** *Moulds, Mycotoxins, Contamination risk, Storage perspective, Storage conditions, Post-harvest handling*

## 1. INTRODUCTION

The multipurpose uses of cluster bean, or guar, (Cyamopsis tetragonoloba L.), in sectors as diverse as food production, medicine, and oil extraction highlight the crop's considerable agricultural significance. Moulds and mycotoxins, however, can weaken cluster bean seeds' quality and safety, endangering the health of humans and other animals. Mycotoxins are produced when agricultural commodities are colonised by moulds, which are omnipresent fungus. When consumed, these mycotoxins can have harmful effects.

Some kinds of mould create secondary metabolites called mycotoxins. The most prevalent ones are aflatoxins, ochratoxins, and fumonisins. A major worry for food safety and public health are these pollutants because of their immunosuppressive, carcinogenic, and mutagenic characteristics. A thorough evaluation of contamination risks in field and storage environments is necessary because cluster bean seeds can be contaminated with moulds and mycotoxins at any point during their life cycle, from growth to harvest to storage.

We measure the presence of moulds and track variations in mycotoxin levels by field surveys performed at different growth stages of cluster beans. Our research also looks into how post-harvest processing, environmental factors, and farming methods affect the likelihood of contamination. In addition, samples taken while storing are examined to learn how mould and mycotoxin contamination might be retained or even intensified under different storage circumstances.

Our goal is to help people understand how to effectively handle cluster bean seeds so that they don't get contaminated with moulds and mycotoxins by combining data from both the field and storage. Good farming practices, optimised post-harvest handling techniques, and improved storage conditions to minimise contamination concerns are all examples of strategies that could be used. Improving seed quality, guaranteeing food safety, and protecting public health are the end goals of this research in the cluster bean production chain.

For the sake of consumer safety and health, it is possible to utilise a variety of degrading procedures in addition to preventative measures to reduce or eradicate mycotoxins from food. Citrinin (CIT) is an effective antibacterial, anticancer, and neuroprotective compound; yet, it is seldom used as a medicine because of its significant nephrotoxicity and genotoxicity (Ordegrul & Azirak, 2004; Chang et al., 2009; Nakajima et al., 2016). Factors such as temperature, relative humidity, fungicide and fertiliser presence, substrate type and nutritional content, geographic location, genetic requirements, insect infestation, and interaction between toxigenic fungus species are some of the variables that affect mould growth and mycotoxin production (Tola & Kebede, 2016). As the most common noninfectious food poisoning, aflatoxins constitute a major danger to public health and a major obstacle to food and nutrition security across Africa. When the fungi Aspergillus flavus and Aspergillus parasiticus produce their toxic metabolites, they belong to a group known as aflatoxins. Soil, air, seeds, and plant remains are all possible places for these toxins to be detected. They could contaminate crops like beans, corn, peanuts, cotton seed, cotton seed meal, and cotton (Bandyopadhyay et al., 2016). Mycotoxin mitigation strategies in African food have received a lot of attention, but because different stakeholders (including farmers, traders, processors, and consumers) learn and absorb information in different ways, the right strategies aren't being put into place to their full potential (Matumba et al., 2016). Because of its strong dependence on weather and climatic variables for agricultural production, sub-Saharan Africa is unfortunately one of the most vulnerable regions to the consequences of global warming (J.H., 2011). According to (Kachapulula et al., 2017), in some places, like Zambia, groundnuts with high mycotoxin contents are mostly caused by weather extremes, such as prolonged drought and extremely hot summers. After the beans have been piled, placed in trays, boxes, or small baskets, the leaves of the plantain are placed on top. Fermentation takes place when they are left in this condition for five to seven days (Fowler & Coutel, 2017). The reduction of aflatoxins confirmed the effectiveness of hermetic technology, particularly the use of hermetic metal silos and hermetic grain bags.

According to the research, hermetic technology could help reduce financial losses due to insect damage. Nyanga (2017) found that conventional storage, metal silos, and hermetic bags all resulted in a weight reduction of 6.11%, 3.54%, and 2.54%, respectively. The purpose of this research is to assess, from a field and storage standpoint, the potential for mould and mycotoxin contamination in cluster bean seeds. To successfully limit contamination risks, we aim to examine the factors that influence contamination during production and post-harvest. This will help us identify crucial areas of intervention. If we want to develop focused measures to guarantee seed quality and food safety in cluster bean seeds, we need to understand the dynamics of mould growth and mycotoxin formation.

## 2. MATERIAL AND METHODS

A thorough methodology was used to evaluate the potential for mould and mycotoxin contamination in cluster bean seeds in both field and storage settings. Cluster bean fields in various parts of the world with varying soil types and climates were sampled by hand. Each field had sampling locations chosen at random to guarantee a representative sample. Gathering samples at several points in the growth cycle allowed us to track the temporal dynamics of contamination, which included flowering, pod formation, and maturity. Throughout the process of collecting samples, great care was taken to ensure that no two samples were ever contaminated. Samples taken from the field were carefully analysed in the lab. Placing seeds onto selective agar media allowed for mould identification, which was then confirmed by assessing colony morphology and, if needed, molecular techniques. We used HPLC or an enzyme-linked immunosorbent test (ELISA) to measure mycotoxin levels, which are both reliable analytical tools. The accuracy of the mycotoxin readings was ensured by using standardized extraction and purification techniques. In order to connect contamination levels with environmental data, such as temperature, humidity, and rainfall, they were collected simultaneously during field sampling. Samples were taken as soon as possible after harvest and then put through controlled environments that mimicked storage conditions. To find out how changing storage conditions like humidity, temperature, and ventilation affected mould growth and mycotoxin production, we did some experimenting. To track any changes in contamination levels while storage was underway, regular sample intervals were set up. To make sure the samples were representative, they were collected methodically from different places inside the storage containers. Samples kept in the lab were analysed in the same way as samples collected in the field. I counted the colonies on agar plates to monitor mould growth, and I assessed mycotoxin levels frequently using the same analytical techniques as I used for field samples. In order to find statistically significant variations in contamination levels across sample locations and storage conditions, we ran the numbers. The associations between environmental variables and pollution levels were further investigated using correlation analysis. The study's reliability and correctness were guaranteed by rigorous adherence to quality control standards. The laboratory equipment was frequently calibrated and validated, and standard operating procedures were followed to the letter. In order to avoid contamination, sterility protocols were rigorously followed when handling and analysing the samples. The danger of mould and mycotoxin contamination in cluster bean seeds was thoroughly assessed using this comprehensive methodology. The examination covered both field and storage views.

## 3. RESULTS AND DISCUSSION

In order to evaluate the potential for mould and mycotoxin contamination in cluster bean seeds, it is essential to determine the presence of aflatoxin B1 in cluster beans (Cyamopsis tetragonoloba L.). This is important from both a field and storage standpoint. Under ideal environmental conditions, moulds like Aspergillus flavus and Aspergillus parasiticus can infect crops like cluster beans and create the deadly mycotoxin aflatoxin B1. One common analytical method for accurately measuring aflatoxin B1 levels in food products is high-performance liquid chromatography, or HPLC.

**A possible flowchart for the procedure is as follows:**

- **Sample Preparation:**
  - Cluster bean seeds that have been harvested are gathered from either fields or places where they are stored.
  - To guarantee consistency, the samples are reduced to a fine powder
  - Aflatoxin B1 is extracted using solvents such as methanol or acetonitrile, and sometimes salts or other additions are added to make the extraction process more efficient.
- **HPLC Analysis:**
  - The examination of aflatoxin B1 is commonly carried out by employing a reverse-phase high-performance liquid chromatography (HPLC) column, which partitions substances according to their hydrophobicity.
  - The detection of aflatoxins B1 is accomplished by employing an appropriate detector, such as a fluorescence detector, which is capable of detecting the natural fluorescence of aflatoxins when they are activated by particular ultraviolet wavelengths of light.
  - In order to obtain quantification, it is necessary to compare the peak area or peak height of the aflatoxin B1 chromatographic peak with a calibration curve that is constructed from recognised standards.
- **Calibration:**
  - In order to generate a calibration curve, it is necessary to establish calibration standards that contain known quantities of aflatoxin B1. These standards are then analysed alongside the samples.
  - As a result of the calibration curve, which establishes a connection between the concentration of aflatoxin B1 and the response of the detector, it is possible to accurately quantify the amounts of aflatoxin present in the samples.
- **Data Analysis:**
  - The determination of the concentration of aflatoxin B1 in every sample is accomplished by extrapolating with respect to the calibration curve.
  - In most cases, the results are commonly stated in measures such as parts per billion (ppb) or micrograms per kilogramme (µg/kg).
- **Risk Assessment:**
  - When comparing the levels of aflatoxin B1 to the regulatory limits established by relevant agencies, such as the Food and Drug Administration (FDA), the results are then analyzed.
  - The seeds are regarded to be contaminated if the levels of aflatoxin B1 surpass specified limits. Consumption of these seeds by either people or animals may result in potential health concerns.
- **Storage Management and Mitigation:**
  - On the basis of the findings, it is possible to establish suitable storage management methods in order to reduce the occurrence of mould development and mycotoxin contamination effectively.

- In order to prevent the growth of mould, it is necessary to take measures such as ensuring enough ventilation, ensuring that moisture levels remain at optimal levels, and employing suitable storage containers or treatments.

When it comes to determining and controlling the risk of mould and mycotoxin contamination in cluster bean seeds, high-performance liquid chromatography (HPLC) analysis is an essential component that contributes to the maintenance of food safety and quality.

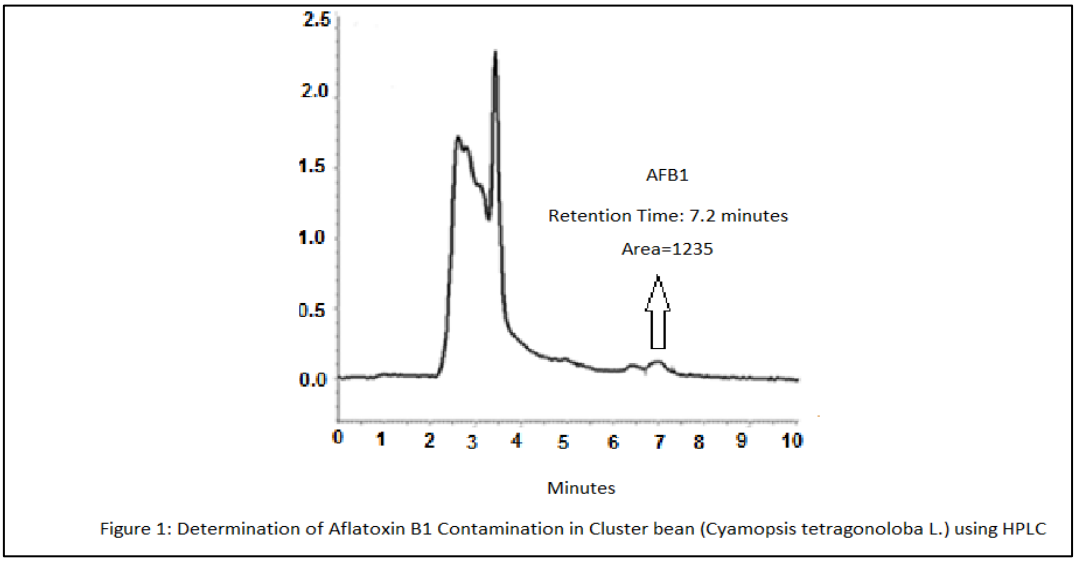


Figure 1: Determination of Aflatoxin B1 Contamination in Cluster bean (Cyamopsis tetragonoloba L.) using HPLC

## 3. CONCLUDING REMARKS

The evaluation of aflatoxin B1 contamination in cluster bean (Cyamopsis tetragonoloba L.) seeds using High-Performance Liquid Chromatography (HPLC) has provided valuable insights into the field and storage perspectives of this important agricultural commodity. Through meticulous analysis and quantification, this study has shed light on the prevalence and levels of aflatoxin B1, a potent carcinogen and mycotoxin, in cluster bean seeds. The findings indicate the importance of monitoring and controlling aflatoxin contamination throughout the production and storage processes to ensure food safety and mitigate health risks associated with consumption. From a field perspective, it is evident that various factors such as environmental conditions, crop management practices, and fungal infestation play significant roles in aflatoxin contamination. Understanding these factors is crucial for implementing effective preventive measures, including proper crop rotation, pest management, and irrigation practices, to minimize fungal growth and aflatoxin production in the field. Furthermore, the study underscores the importance of post-harvest handling and storage practices in mitigating aflatoxin contamination. Poor storage conditions, such as high moisture levels and inadequate ventilation, can exacerbate fungal growth and toxin production in stored cluster bean seeds. Therefore, implementing proper drying, cleaning, and storage techniques, along with regular monitoring of aflatoxin levels, is essential for preserving seed quality and ensuring food safety. The use of HPLC as an analytical tool has proven to be reliable and accurate for the detection and quantification of aflatoxin B1 in cluster bean seeds. Its sensitivity and specificity allow for precise measurements, enabling researchers and food safety authorities to assess the extent of contamination and enforce regulatory standards. In conclusion, the findings from this evaluation highlight the importance of comprehensive strategies encompassing both pre-harvest and post-harvest interventions to mitigate aflatoxin contamination in cluster bean seeds. By implementing integrated approaches that address the various stages of production and storage, stakeholders can safeguard both human health and economic interests associated with

cluster bean cultivation. Continued research efforts and collaborative initiatives are needed to develop and disseminate best practices for aflatoxin management in cluster bean production systems, ultimately ensuring a safer and more sustainable food supply.

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