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(Review Article)

Mycotoxin contamination in millets: Microbiological insights and quality implications

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Abstract

This study is formulated to review various aspects of mycotoxin contamination in millets. It provides an in-depth examination of the various mycotoxins that commonly afflict millet crops, including aflatoxins, fumonisins, deoxynivalenol (DON), and zearalenone. These toxic secondary metabolites are produced by molds, predominantly *Aspergillus* and *Fusarium* species, under specific environmental conditions. Mycotoxin contamination in millets is a pressing concern that impacts food safety, human health, and the global agricultural landscape. Millets, revered for their resilience and nutritional value, are integral to the diets of millions worldwide. However, their susceptibility to mycotoxin contamination poses significant threats to both crop yield and the quality of millet-based products. On the other hand the threat of mycotoxin contamination in millets underscores the necessity of a multidisciplinary approach that encompasses microbiology, agriculture, food science, and regulatory compliance. By delving into microbiological insights surrounding mycotoxin contamination and examining its quality implications, this study seeks to contribute to safer, higher-quality millet production and consumption. It emphasizes the importance of collaboration between researchers, policymakers, and the agricultural community to address this critical concern and promote the sustainable cultivation of millets on a global scale for ensuring food safety and facilitating international trade. Consequently, the study highlights the importance of harmonizing regulations to safeguard public health and promote the global trade of millet-derived products.

Keywords: Millets; Mycotoxin; Contamination; Food Safety; Cereal and Grains; International Trade; Mitigation

1. Introduction

Mycotoxin contamination in millets is a growing concern worldwide, far-reaching implication for food safety, human health, and the agricultural sector, impacting both food safety and nutritional quality [1]. Millets, being drought-tolerant and nutritionally rich grains, have gained popularity as staple foods in many regions. However, the presence of mycotoxins, toxic secondary metabolites produced by microorganisms, poses a significant threat to millet quality and health hazards to consumers [2]. Mycotoxins are naturally occurring contaminants that can infect various varieties of millets, during growth, harvesting, and storage. Microbiological research has been pivotal in understanding the dynamics of mycotoxin-producing fungi, their interactions with millet plants, and the mechanisms underlying mycotoxin production. Mycotoxins can infest crops under specific environmental conditions. These toxins can accumulate in grains, posing severe health risks [3]. Millets, a group of small-seeded, highly nutritious cereal grains, are traditionally grown and consumed in many regions around the world. They are valued for their resilience in diverse environmental conditions and their role in providing food security for millions of people. However, the presence of mycotoxins in millets poses a substantial threat to both crop yield and the safety of millet-based products [4]. India is one of the largest producers of millets in the world, with various types of millets being cultivated across the country. The major millet varieties produced in India include pearl millet (bajra), finger millet (ragi), foxtail millet (kangni), and little millet (kutki) [5]. Here's a rough estimate of millet production in India as of 2020:

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- **Pearl Millet (Bajra)**: India is the largest producer of pearl millet in the world. It is primarily grown in states like Rajasthan, Gujarat, and Haryana. In 2020, India produced approximately 10 million metric tons of pearl millet [6]. (Graph-01).
- **Finger Millet (Ragi)**: Finger millet is commonly grown in southern India, particularly in Karnataka, Tamil Nadu, and Andhra Pradesh. In 2020, India produced around 3 million metric tons of finger millet [6]. (Graph-02)
- **Jowar (Sorghum):** Jowar is one of the principal millets cultivated in India, largely grown in Maharashtra, Karnataka, and Madhya Pradesh. In 2020, India produced approximately 4.5 million metric tons of jowar [6]. (Graph-03)
- **Foxtail Millet (Kangni)**: Foxtail millet is grown in various states across India. In 2020, India produced approximately 1.5 million metric tons of foxtail millet [7].
- **Little Millet (Kutki)**: Little millet is cultivated in states like Karnataka, Tamil Nadu, and Maharashtra. In 2020, India produced roughly 0.7 million metric tons of little millet [8]. **(Table-01).**

Further, millet production occurs in many countries around the world, and the total production can vary from year to year. Millets are grown in regions with diverse climates, from arid areas to more temperate zones. While India is a significant producer, other countries also contribute to global millet production [9].

- **Africa**: Several African countries, including Nigeria, Niger, Mali, Burkina Faso, and Sudan, are major millet producers. Pearl millet is particularly important in West Africa, while finger millet is widely grown in East Africa [10].
- **China**: China is one of the largest producers of millets globally, including foxtail millet and proso millet [9].
- **United States**: The United States also produces millets, including proso millet, which is primarily grown in states like Colorado and Nebraska [11].
- **Russia**: Millets, particularly proso millet, are grown in parts of Russia [12]. **(Table-02).**

The quality implications of mycotoxin contamination in millets extend beyond economic losses and health concerns. Research's will shed light on the impact of mycotoxins on millet grain quality, including their effects on nutritional value, sensory attributes, and suitability for processing [13]. Moreover, this exploration will touch upon the regulatory framework and standards governing mycotoxin levels in millet products, both at the national and international levels. Understanding these regulations is essential for ensuring compliance and safeguarding food safety [14].

Thus, the threat of mycotoxin contamination in millets is a pressing issue that requires a multidisciplinary approach encompassing microbiology, agriculture, food science, and regulatory compliance [15]. By delving into the microbiological insights surrounding mycotoxin contamination and examining its quality implications, this study aims to contribute to the development of strategies for safer, higher-quality millet production and consumption. Additionally, it underscores the importance of collaboration between researchers, policymakers, and the agricultural community to address this critical concern and promote the sustainable cultivation of millets worldwide [16].

1.1. Mycotoxin-Producing Fungi in Millets

Understanding the diversity of mycotoxin-producing fungi in millet crops is essential. Mycotoxin-producing fungi in millets are microorganisms that belong to various genera and species such as *Aspergillus, Penicillium,* and *Fusarium* and have the ability to synthesize mycotoxins like aflatoxins, fumonisins, and trichothecenes which are toxic secondary metabolites [17]. These fungi are primarily responsible for the contamination of millet crops with mycotoxins. Subsequently, the identity and characteristics of these fungi is crucial for managing and mitigating mycotoxin contamination in millets [18]. **(Table-03)**

Here, some of the common mycotoxin-producing fungi found in millets:

1.1.1. Aspergillus spp.

- ***Aspergillus flavus*****:** This fungus is a major producer of aflatoxins, particularly aflatoxin B1, which is highly carcinogenic and poses a severe health risk to humans and animals. *A. flavus* is a common contaminant of millet crops, especially when environmental conditions, such as high temperatures and moisture, are conducive to its growth [19].
- ***Aspergillus parasiticus*****:** Like *A. flavus, A. parasiticus* is another significant producer of aflatoxins. It can infect millet grains and produce aflatoxin B1 and other aflatoxins when conditions are favorable for mold growth [20].
- ***Aspergillus niger****:* Unlike *A. flavus* and *A. parasiticus*, *A. niger* is generally considered less toxigenic, but it is known to produce ochratoxin A and fumonisins under certain conditions. It is one of the most common storage

fungi associated with millet grains and can cause black discoloration of kernels, leading to deterioration in both quality and nutritional value [21]. (Table-04) (Fig-01).

1.1.2. Fusarium spp.

- ***Fusarium graminearum:*** This fungus produces mycotoxins such as deoxynivalenol (DON) and zearalenone, which can contaminate millets. DON is known for its negative impact on human and animal health, particularly in high concentrations [22].
- ***Fusarium verticillioides:*** This fungus is a primary producer of fumonisins, particularly fumonisin B1, which can contaminate millets. Fumonisins can cause various health issues in humans and livestock, including neural tube defects and esophageal cancer.
- ***Fusarium proliferatum:*** Another producer of fumonisins, *F. proliferatum,* can also infect millet grains and contribute to fumonisin contamination [23]. **(Table-05**) (Fig-02)

1.1.3. Penicillium spp.

- ***Penicillium verrucosum:*** This fungus is responsible for the production of ochratoxin A, a mycotoxin that can contaminate millet grains. Ochratoxin A has been associated with nephrotoxicity and carcinogenicity in humans [24]. (Fig-03)
- ***Alternaria spp.:***
- ***Alternaria alternata:*** Some *Alternaria* species can produce mycotoxins such as alternariol and alternariol monomethyl ether (AME). These mycotoxins can contaminate millets and are associated with a range of toxic effects in animals [25]. (Fig-04)

1.1.4. Claviceps spp.

- ***Claviceps purpurea:*** This fungus is responsible for the production of ergot alkaloids. While millets are not the primary hosts for Claviceps, they can become contaminated if they grow in proximity to ergot-infected grasses. Ergot alkaloids can cause various health issues in humans and livestock [26]. (Fig-05)

1.2. Environmental Factors Influencing Mycotoxin Contamination:

Several microbiological research has highlighted the role of environmental factors, including temperature, humidity, and moisture content, in promoting mycotoxin contamination in millets. In order to forecast and prevent mycotoxin outbreaks, it is essential to comprehend the microbial ecology of millet fields and storage conditions [27]. (Fig-06). Some of the key environmental factors that influence mycotoxin contamination are given blow:

- **Temperature:** Temperature is a significant factor influencing mycotoxin production. Different mycotoxins have specific temperature ranges at which they are most active. For example, aflatoxin production by *Aspergillus* species is favored by high temperatures, typically ranging from 25°C to 35°C (77°F to 95°F) [28]. In contrast, fumonisins production by *Fusarium* species occurs in a slightly cooler range, around 20°C to 30°C (68°F to 86°F). Therefore, variations in temperature during millet growth, harvest, and storage can impact mycotoxin contamination.

- **Moisture Content:** Moisture content in millet crops is a critical factor in mycotoxin contamination. Fungal growth and mycotoxin production require a certain level of moisture. Aflatoxins, for instance, thrive in environments with high relative humidity and moisture levels in the range of 15% to 30%. Proper drying of millet grains to a safe moisture level (typically below 12%) before storage is essential to prevent mycotoxin contamination [29].

- **Humidity:** Relative humidity (RH) in the environment can promote or inhibit mycotoxin production. High RH levels can create favorable conditions for mold growth and mycotoxin synthesis. Additionally, temperature and RH interact, with warm temperatures increasing the moisture-holding capacity of the air. Therefore, regions with high temperatures and humidity are at a higher risk of mycotoxin contamination in millet crops [30].

- **Rainfall and Irrigation:** Weather patterns, including rainfall and irrigation practices, significantly impact millet crops' exposure to moisture. Excessive rainfall and improper irrigation can lead to waterlogged soils and increased moisture in the field, creating conditions conducive to mold growth. Adequate drainage and irrigation management are essential to minimize the risk of mycotoxin contamination [31].

- **Drought Stress:** Conversely, drought stress can also influence mycotoxin contamination. Millet plants subjected to drought stress may produce stress-related hormones that can enhance susceptibility to fungal infections. Drought conditions can lead to cracks and physical damage in millet grains, providing entry points for molds [32].

- **Crop Residue Management:** Leftover crop residues in the field from previous harvests can harbor mycotoxin-producing molds. Proper disposal or incorporation of crop residues can help reduce the inoculum source for mycotoxins in the current crop [30].
- **Insect Damage:** Insects can introduce mycotoxin-producing fungi into millet crops and facilitate their spread. Insect-damaged millet grains are also more susceptible to mold infestations, increasing the risk of mycotoxin contamination [33].
- **Storage Conditions:** Post-harvest storage conditions, including temperature and humidity in storage facilities, play a crucial role in mycotoxin development. Improperly stored millet grains can provide an environment for ongoing mold growth and mycotoxin production [23].

1.3. Microbiome-Mycotoxin Interactions

The millet microbiome plays a significant role in mycotoxin contamination. Microbial communities in the rhizosphere, endosphere, and phyllosphere can influence the presence and activity of mycotoxin-producing fungi. Researches related to microbial interactions in millet fields can help to identify potential biocontrol agents or strategies for reducing mycotoxin contamination. Since, microbiome-mycotoxin interactions in millets refer to the complex relationships between the microorganisms inhabiting the millet crop and the production or degradation of mycotoxins [34]. The millet microbiome consists of diverse communities of bacteria, fungi, viruses, and other microorganisms, both beneficial and harmful [35]. These microorganisms can influence mycotoxin contamination in millets through various mechanisms. Understanding these interactions is essential for managing mycotoxin contamination effectively [13]. (Fig-07)

Here are the key aspects of microbiome-mycotoxin interactions in millets:

1.3.1. Antagonistic Microorganisms:

- **Biocontrol Agents:** Some beneficial microorganisms, known as biocontrol agents, have the ability to inhibit the growth and activity of mycotoxin-producing fungi. For example, certain bacterial and fungal species can produce antimicrobial compounds that suppress the growth of mycotoxin-producing molds.

These biocontrol agents can be applied as biological pesticides or incorporated into the soil to reduce mycotoxin contamination in millet crops [36]. (Fig-08)

1.3.2. Competitive Exclusion

- **Microbial Competition:** The millet microbiome is a complex ecosystem where microorganisms compete for resources. In some cases, non-toxic or less harmful molds and bacteria can out compete mycotoxin-producing fungi for nutrients and space. This competition can reduce the prevalence of mycotoxin-producing molds in the millet crop [37].

1.3.3. Degradation and Detoxification

- **Microbial Degradation:** Some microorganisms in the millet microbiome possess enzymatic capabilities that allow them to break down mycotoxins into non-toxic or less toxic metabolites. This process, known as microbial degradation, can occur in the field or during post-harvest storage, potentially reducing mycotoxin levels in millets [38].

1.3.4. Mycotoxin Production by Microbiota

- **Secondary Metabolite Production:** While the focus is often on mycotoxin-producing molds, certain bacteria and fungi within the millet microbiome can also produce secondary metabolites with toxic properties. These metabolites can contribute to overall mycotoxin contamination in millets [39].

1.3.5. Modulation of Host Plant Defense

- **Plant-Microbe Interactions:** The millet microbiome can influence the defense mechanisms of millet plants. Some beneficial microorganisms can induce plant defense responses, making the crop less susceptible to mycotoxin-producing molds. Conversely, pathogenic microorganisms may weaken plant defenses, increasing susceptibility to fungal infections and mycotoxin contamination [3].

1.3.6. Environmental Factors

- **Microbiome Responses to Environmental Conditions:** Environmental factors, such as temperature, humidity, and soil characteristics, can shape the composition and activity of the millet microbiome. Changes in these factors can impact the prevalence and activity of mycotoxin-producing fungi and their antagonistic microorganisms [40].

1.4. Mitigation Strategies

Microbiological studies have contributed to the development of various mitigation strategies for mycotoxin control in millets. These include the use of biocontrol agents, the application of competitive exclusion principles, and the development of resistant millet varieties. Mitigating mycotoxin contamination in millets is essential to ensure the safety and quality of millet-based products [18]. Mycotoxins can have adverse effects on human and animal health, making it crucial to implement strategies to prevent their formation and reduce their presence in millet crops and products [34]. Here are key mitigation strategies for mycotoxin contamination in millets:

1.4.1. Good Agricultural Practices (GAPs)

- **Crop Rotation:** Implementing crop rotation helps reduce the buildup of mycotoxin-producing fungi in the soil. Rotate millets with non-host crops to break the disease cycle [41].
- **Selection of Resistant Varieties:** Choose millet varieties that are less susceptible to mycotoxin-producing molds. Breeding programs can develop resistant cultivars.
- **Timely Harvesting:** Harvest millet crops at the optimal maturity stage to minimize exposure to environmental conditions conducive to mold growth.
- **Proper Drying:** Ensure millet grains are thoroughly dried before storage, reducing moisture content to safe levels (typically below 12%). Use sun drying or mechanical drying methods [42].

1.4.2. Effective Pest and Weed Management

- **Insect Control:** Implement integrated pest management (IPM) practices to control insect pests that can damage millet grains and create entry points for mycotoxin-producing molds [41].
- **Weed Control:** Keep millet fields weed-free to reduce competition for nutrients and moisture, which can favor mold growth [43].

1.4.3. Storage and Post-Harvest Management:

- **Clean and Dry Storage Facilities:** Maintain clean, dry, and well-ventilated storage facilities to prevent moisture buildup and inhibit mold growth.
- **Proper Grain Handling:** Handle millet grains gently to minimize physical damage that can increase susceptibility to fungal infection [44].
- **Regular Inspection:** Monitor stored millet grains regularly for signs of mold growth and mycotoxin contamination. Remove contaminated grains promptly to prevent further spread [41].

1.4.4. Biological Control

- **Use of Biocontrol Agents:** Apply beneficial microorganisms, such as certain bacterial and fungal species, that can out compete mycotoxin-producing molds in the field or during storage [45].

1.4.5. Aflatoxin-Specific Strategies:

- **Atoxigenic Strains:** Use atoxigenic strains of *Aspergillus flavus*, which do not produce aflatoxins, as biocontrol agents to reduce aflatoxin contamination in millets [46].

1.4.6. Pre-Harvest and Post-Harvest Chemical Control:

- **Fungicides and Preservatives:** In some cases, application of approved fungicides or preservatives can help reduce fungal growth and mycotoxin contamination. However, this approach should be used judiciously and in compliance with local regulations [47].

1.4.7. Mycotoxin Testing and Monitoring:

- **Regular Testing:** Implement mycotoxin testing programs to assess the contamination levels in millet batches before processing or consumption. Discard or segregate millet lots with high mycotoxin levels [29].

- **Monitoring Tools:** Utilize rapid and cost-effective monitoring tools, such as lateral flow devices, to check mycotoxin levels in the field or during storage [48].

1.5. Education and Training

- **Farmer and Stakeholder Awareness:** Educate farmers, processors, and other stakeholders about the risks associated with mycotoxins and the importance of implementing mitigation strategies [49].

1.5.1. Regulatory Compliance

- **Adhere to Regulations:** Comply with national and international regulations and standards governing mycotoxin levels in millet products [29].

1.5.2. Research and Innovation

- **Innovative Technologies:** Explore and develop innovative technologies and biotechnological interventions to reduce mycotoxin contamination in millets [46].

2. Conclusion

In concluding remarks, the main aim of this review article is to focus on the threats in millets due to mycotoxin contamination. It remains a significant challenge in the millet industry in various prospective like production and consumption demands in domestic as well as export market. Microbiological research is instrumental in understanding the microbiome-mycotoxin interactions, environmental factors, and mitigation strategies that can help safeguard millet quality. This review article underscores the multifaceted nature of mycotoxin contamination in millets and the vital role of microbiological research in addressing this global concern. It highlights the urgent need for comprehensive strategies, regulatory compliance, and ongoing research efforts to safeguard millet crops, protect consumer health, and support the sustainable cultivation of millets worldwide.

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Supplementary Tables and Figures

Table 1 Major Millets Production in India (2020 estimates)

Millet Variety	Major States	Production (MMT)	Reference
Pearl Millet (Bajra)	Rajasthan, Gujarat, Haryana	~10.0	Rakshit et al., 2023
Finger Millet (Ragi)	Karnataka, Tamil Nadu, Andhra Pradesh	~3.0	Rakshit et al., 2023
Foxtail Millet (Kangni)	Andhra, Karnataka, Telangana	~1.5	Ramesh et al., 2020
Little Millet (Kutki)	Karnataka, Maharashtra, Tamil Nadu	~0.7	Tagade et al., 2023

Table 2 Global Millet Production (Selected Countries)

Country	Major Millet Types	Production Status	Reference
India	Pearl, Finger, Foxtail, Little	Largest producer worldwide	Chandra et al., 2021
Nigeria & Niger	Pearl Millet	Key staple crop	Meena et al., 2021
China	Foxtail, Proso	One of largest global producers	Chandra et al., 2021
USA (Colorado, Nebraska)	Proso Millet	Niche but growing	Verma et al., 2018
Russia	Proso Millet	Moderate production	Das et al., 2019

Table 3 Microscopic Comparison: *Aspergillus parasiticus* vs *A. flavus* and *A. niger*

Feature	*A. parasiticus*	*A. flavus*	*A. niger*
Colony Color	Dark green	Yellow-green	Black or dark brown
Conidiophore Length	Short (~400 µm)	Longer	Long and smooth
Vesicle Size	Small (~30 µm)	Larger	Large and globose
Phialide Arrangement	Direct on vesicles	Uniseriate or biseriate	Biseriate
Conidia	Rough, spherical, dark green	Rough, spherical, 3–6 µm	Rough, brown to black, 4–5 µm
Hyphae	Septate, hyaline	Septate, hyaline	Septate, hyaline
Toxin Production	Aflatoxins B1, B2, G1, G2	Aflatoxins B1, B2; cyclopiazonic acid	None
Growth Temperature	12–42 °C (optimal ~32 °C)	Up to 48 °C	Up to 40 °C
Industrial Use	Limited	Limited	Citric acid, enzyme production

Table 4 Common Mycotoxin-Producing Fungi in Millets

Fungal Species	Mycotoxin	Health Impacts
Aspergillus flavus	Aflatoxin B1	Carcinogenic (Liver cancer)
Fusarium verticillioides	Fumonisin B1	Neural tube defects, esophageal cancer
Fusarium graminearum	DON, Zearalenone	Vomiting, reproductive toxicity
Penicillium verrucosum	Ochratoxin A	Nephrotoxicity, carcinogenicity
Alternaria alternata	Alternariol, AME	Cytotoxic, genotoxic
Claviceps purpurea	Ergot Alkaloids	Ergotism, convulsions

Table 5 Comparative analysis for Microscopic Features of *Fusarium spp*.

Fusarium Species	Microscopic Morphology	Distinctive Features
Fusarium graminearum	Produces abundant *macroconidia* (sickle-shaped, 3–5 septa); microconidia are rare or absent	Large, multi-septate, canoe-shaped macroconidia; perithecia often observed
Fusarium verticillioides	Produces abundant *microconidia* (oval to club-shaped, usually 1–2 celled) arranged in long chains; macroconidia slender with few septa	Long chains of microconidia (diagnostic feature)
Fusarium proliferatum	Produces both microconidia (oval, single-celled) and macroconidia (slender, multi-septate); microconidia often formed in false heads	Abundant microconidia in false heads at phialide tips

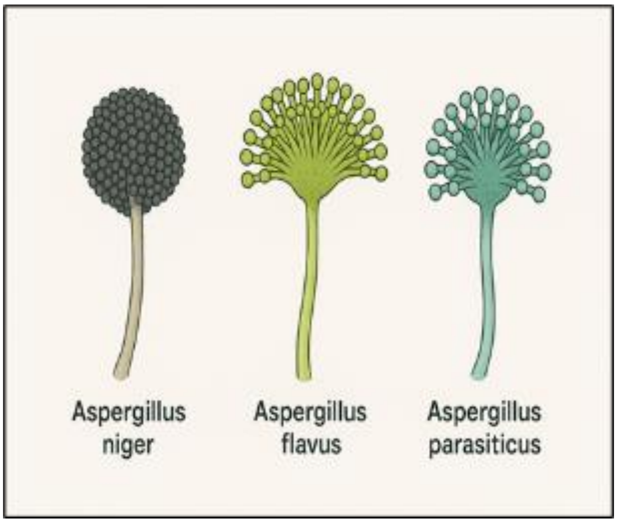


Figure 1 *Aspergillus spp.*

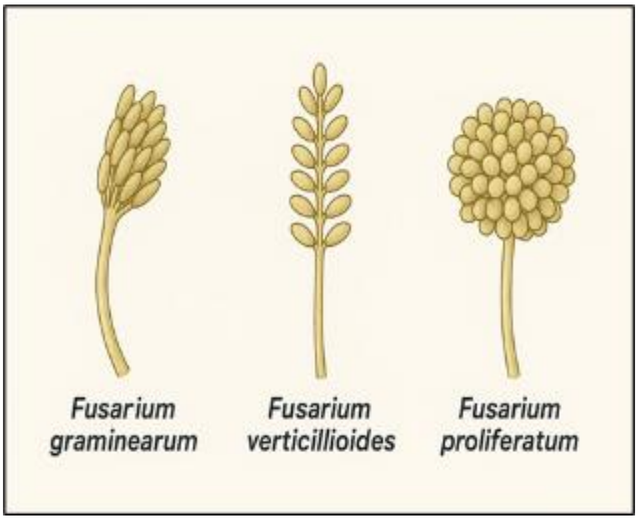


Figure 2 *Fusarium spp.*

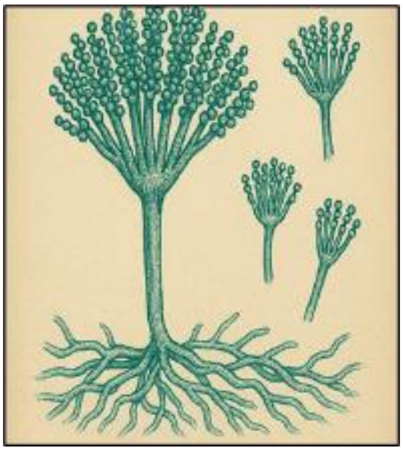


Figure 3 *Penicillium spp.*

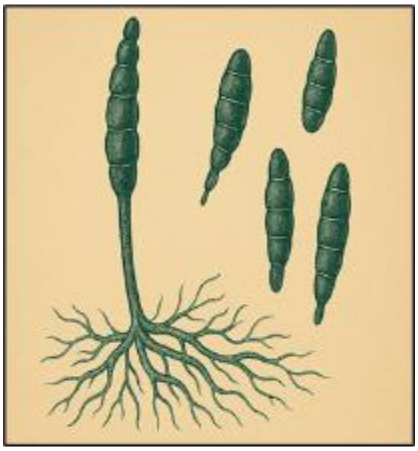


Figure 4 *Alternaria spp*

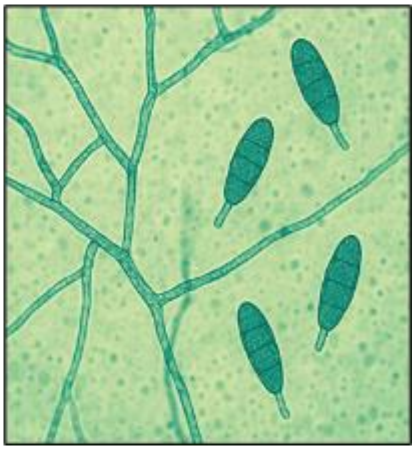


Figure 5 *Claviceps spp.*

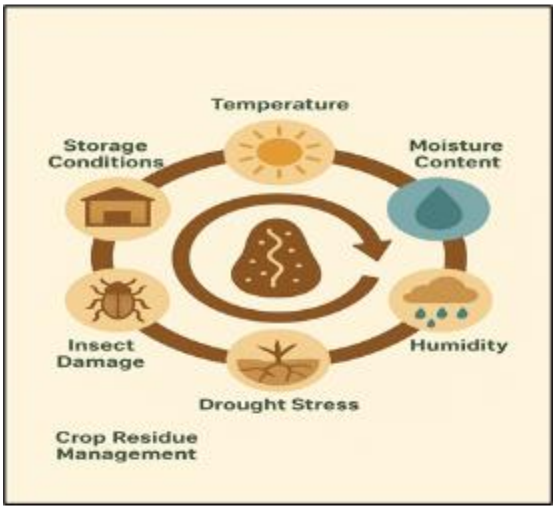


Figure 6 Factors Influencing Mycotoxin

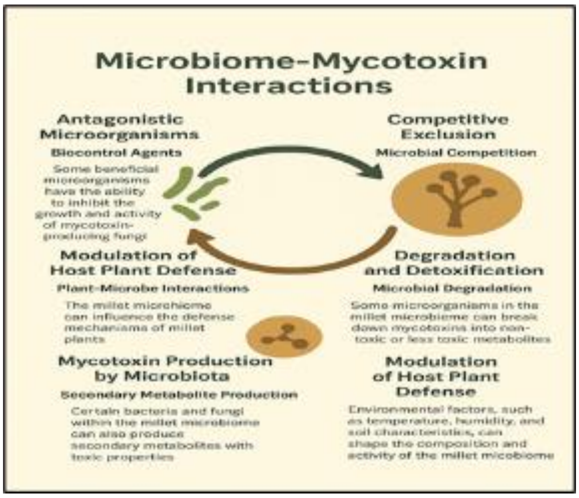


Figure 7 Microbiome-Mycotoxin Interactions

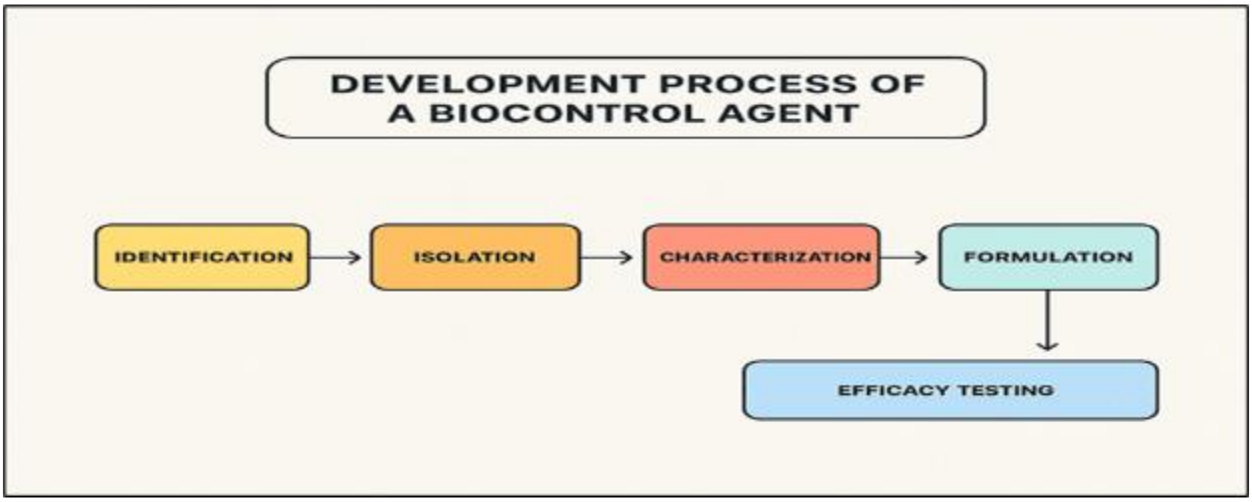
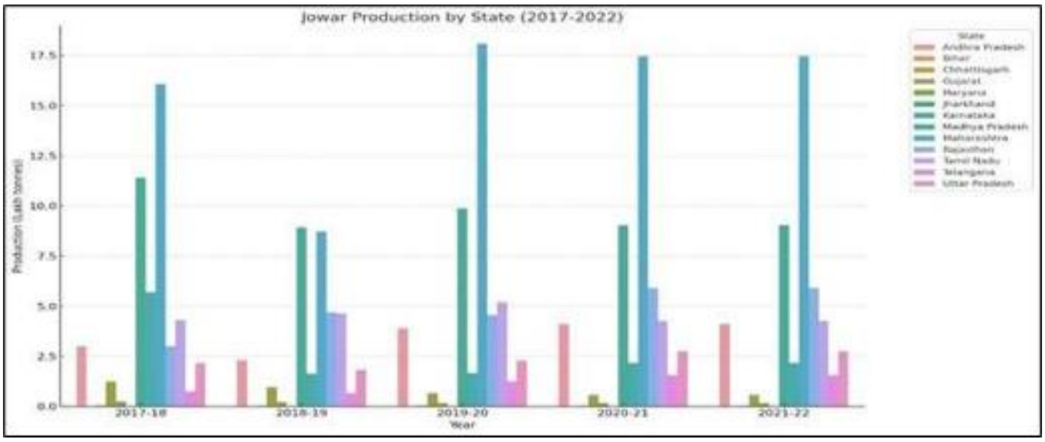
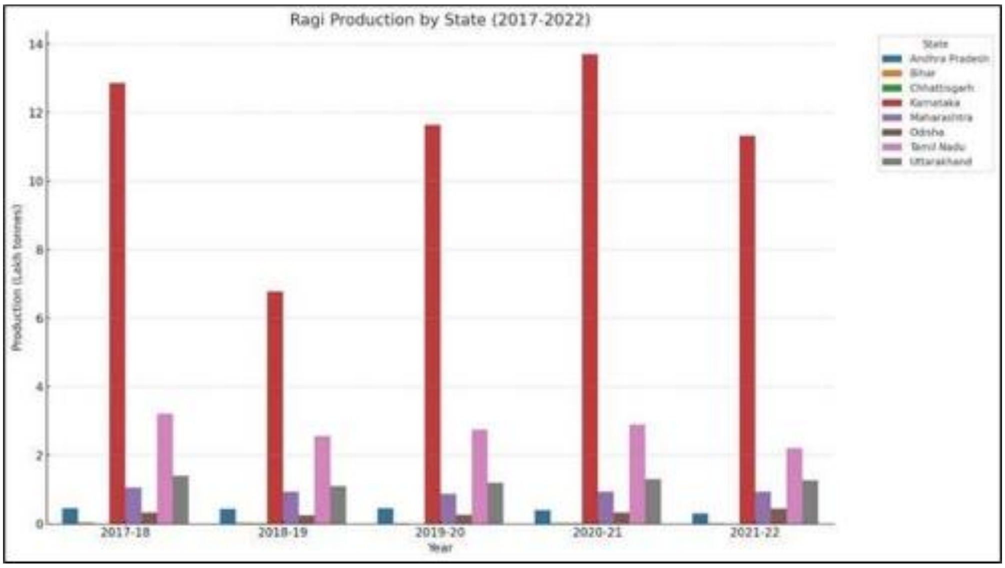


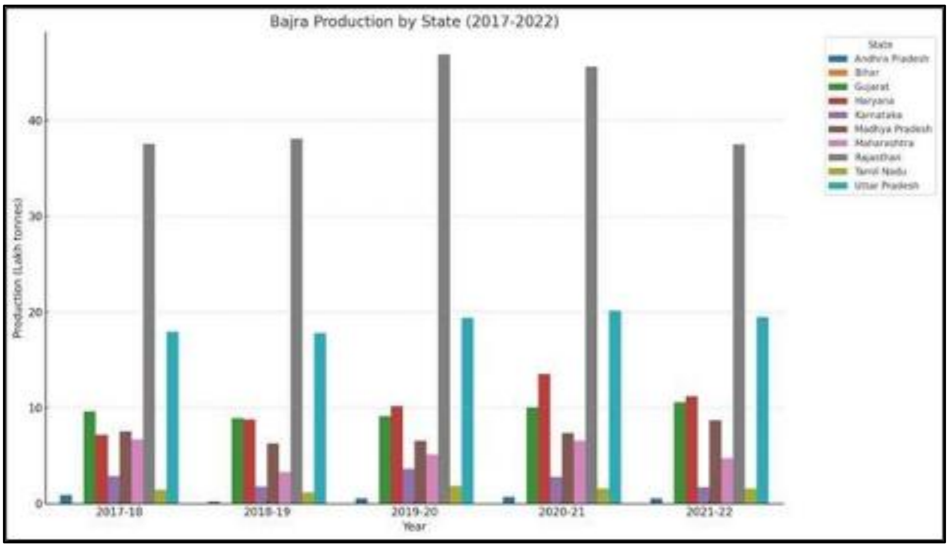
Figure 8 Development Process of Biocontrol Agents



Graph 1 Jowar production data (state wise- 2017-2022)



Graph 2 Ragi production data (state wise- 2017-2022)



Graph 3 Bajra production data (state wise- 2017-2022)