SUMMARY

of the doctoral thesis entitled:

MOLECULAR CHARACTERIZATION OF MYCOTOXIGENIC FUNGI ISOLATES FROM ANIMAL FEED

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Feeds are essential in farm animal nutrition but are often affected by fungal contamination and the production of mycotoxins, dangerous toxins generated by fungi species such as Aspergillus, Fusarium, and Penicillium. These fungi can colonize fodder during harvesting, transportation, and storage, promoting mycotoxin production under unfavorable climatic conditions and high humidity.

Mycotoxins adversely affect the health of animals and birds, causing various harmful effects from reduced growth performance to immunosuppression and mortality. Consumption of contaminated fodder can lead to the bioaccumulation of mycotoxins in animal-derived products, posing a significant risk to human health.

Monitoring and controlling mycotoxin contamination is crucial for protecting public health and animal welfare, essential in international initiatives for food safety and global health. In Africa, including Sudan, climatic conditions and suboptimal agricultural practices increase the risks of mycotoxin contamination in fodder, necessitating urgent interventions to manage these issues.

The doctoral thesis titled " Molecular Characterization Of Mycotoxigenic Fungi Isolates From Animal Feed" represents a comprehensive study of fungi contaminating fodders. The work analyzes four sample types: sorghum grains, shelled peanuts, peanut flour, and wheat bran. The project involves processes of isolation, purification, and cultivation of these fungi in culture media, as well as their morphological and molecular characterization and identification. Additionally, the thesis proposes experiments and solutions to inhibit the growth of these fungi under laboratory conditions, thereby ensuring healthier biological material.

The structure of the doctoral thesis comprises seven chapters divided into two sections. The first section includes a concise introduction to the addressed issue and a literature review of the current research status in the thematic field. The second section encompasses the author's own research.

Chapter I focuses on the main genera and species of mycotoxigenic fungi and their identification methods.

This chapter presents the biological and ecological characteristics of mycotoxigenic fungi from the genera Aspergillus and Fusarium, including their molecular features focusing on genes involved in mycotoxin production and modern molecular identification methods. The chapter provides an overview of mycotoxin and/or fungi contamination in Africa, specifically in Sudan, highlighting regional specificities and their impact on public health and food security in this region.

Chapter II concentrates on treatment methods for reducing mycotoxins in fodder, including physical, chemical, and biological approaches. These methods are crucial for improving food safety and farm animal performance. In the thesis summary, the importance of continuing research and development in this field is emphasized to optimize the efficiency of these techniques and adapt them to specific production conditions.

In Chapter III, isolation, purification, and cultivation of contaminating fungi from various types of fodder in Sudan were carried out, including sorghum, peanuts, wheat bran, and peanut flour. The primary

objective was to identify mycotoxigenic fungi and associated mycotoxins. Specific isolation and identification methods enabled the acquisition of multiple fungal strains, including Aspergillus, Penicillium, Fusarium, Cladosporium, and Alternaria. Predominance was observed in the genus Aspergillus, especially in peanut samples. In total, 32 fungal strains were isolated and identified, with most belonging to the genus Aspergillus (87.37%), including 15 strains from section Nigri, 10 from section Flavi, and 2 associated with section Nidulans. The remaining isolates included Penicillium sp. (1 strain), Fusarium sp. (2 isolates), Cladosporium sp. (1 strain), and Alternaria sp. (1 strain). The results highlighted that the majority of potentially mycotoxigenic isolates, especially from the Aspergillus Flavi and Nigri groups, were obtained from peanuts, both from kernels and flour. The study also underscored the importance of the Flavi group through detailed microbiological analyses confirming the presence of A. flavus in the analyzed samples. These findings emphasize the need for rigorous monitoring and effective strategies for managing risks associated with mycotoxin contamination in animal feed and, consequently, human consumption in Sudan.

In Chapter IV, the study on filamentous fungi in fodder utilized advanced methods such as DNA extraction, species-specific primer amplification, and ITS-RFLP for species identification, including Aspergillus, A. flavus, and A. niger. The results highlighted the predominance of these species and the presence of others such as Penicillium, Fusarium, and Alternaria in fodder samples. Genomic evaluations with RAPD and SCoT revealed significant diversity in Aspergillus, highlighting their adaptability in fodder environments and contributing to the optimization of molecular analysis techniques.

Chapter V evaluated the mycotoxigenic potential of fungal isolates from fodder, focusing on Aspergillus and Penicillium. Methods included the cultivation on mycotoxin detection media such as aflatoxins and the use of TLC and PCR to identify genes involved in their biosynthesis. The results showed that all strains of Aspergillus flavus produced aflatoxins on various culture media, with the coconut milk method being efficient for their detection.

TLC identified AFB1, AFB2, and AFG1 in multiple strains, with AFB1 predominating in A. flavus D3-2. For Penicillium polonicum A3-4 and Aspergillus niger, production of ochratoxin and patulin was confirmed through TLC and PCR. Genes involved in aflatoxin biosynthesis were identified in A. flavus D3-2, highlighting its mycotoxigenic potential. In conclusion, the study demonstrated that isolates of Aspergillus flavus and Penicillium polonium isolated from fodder in Sudan exhibited mycotoxigenic potential, with variable production of aflatoxins, ochratoxin, and patulin. The results suggest the need for rigorous monitoring and effective control strategies to minimize risks associated with mycotoxin contamination in food chains in this region.

Chapter VI investigated the use of lactic acid bacteria to reduce mycotoxin contamination in fodder. Their effectiveness in inhibiting fungi development, including Aspergillus responsible for aflatoxin production, was analyzed. Experimental methods included aflatoxin detection through thin-layer chromatography (TLC). The results demonstrated that adding strains of lactic acid bacteria to fodder led to the absence of aflatoxins produced by Aspergillus flavus A3-5, contrasting with untreated fodder. This promising approach can contribute to reducing risks to animal health and food safety, with the potential to minimize economic losses associated with fodder contamination.

Chapter VII, General Conclusions, highlighted the following important aspects to be included in the summary:

- Identification and isolation of mycotoxigenic fungi from various raw materials used in animal feed in Sudan, including sorghum, peanuts, wheat bran, and peanut flour, emphasizing the predominance of the genus Aspergillus and species from the Flavi and Nigri sections, known for their ability to produce aflatoxins.
- The importance of advanced methodologies such as ITS-RFLP and nucleotide sequencing in accurately identifying mycotoxigenic fungal species contributes to a more precise assessment of contamination risks.
- The demonstrated effectiveness of lactic acid bacteria in reducing aflatoxin contamination in fodder,

suggests that they could represent a viable and sustainable solution in the animal feed industry.

- Recommendations for implementing a rigorous monitoring system for fodder quality, including using lactic acid bacteria in production processes and educating farmers to minimize mycotoxin contamination risks.

These aspects underline the impact of research on controlling and preventing mycotoxin contamination in animal feed, with significant implications for public health and food safety. Implementing these recommendations could improve agricultural practices and regulations, reducing risks associated with consuming contaminated fodder in Sudan and similar regions.

The experiments described in this thesis were conducted at the Molecular Biology Laboratory of the Faculty of Biotechnology within USAMV Bucharest.

The bibliography of this doctoral thesis includes 118 specialized literature sources, both national and international, consisting of books, articles, diploma works, journals, doctoral theses, and accredited web pages. The research results obtained over the years and during the doctoral stage have been published in scientific journals indexed in ISI and BDI, according to the attached publication list.