Impact of Toxigenic Fungi: An Economic and Health Obstruction to Wellness in Mozambique

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Impact of Toxigenic Fungi: An Economic and Health Obstruction to Wellness in Mozambique

T. Fernandes, J. Ferrão, I. Chabite, J. Guina, C. Garrine, and V. Bell

ABSTRACT

Food safety is a major concern worldwide. Food-crop contamination by fungi and mycotoxins is a common occurrence causing persistent exposure that raises critical health problems and economic losses. Food and feeds are frequently tainted by multiple contaminants, such as trace elements, heavy metals, dioxins, pesticides, and mycotoxins. Most African countries lack the ability to enforce international food safety regulations and face frequent rejection of exportable raw food materials leading to financial burden and increased intramural consumption of contaminated products. The literature on mycotoxins is extremely vast, investigating or reporting cellular mechanisms and toxicity, associated pathology and animal performance, effects of these compounds on general malnutrition and on human health. However, different sampling and analytical methods for research has hindered progress, data collection and interpretation. Innovative and promising commercial solutions of technological biocontrol have been approved in few African countries but may not be the sole and long-lasting solution for the management of mycotoxins. We describe an economic burden in Mozambique of naturally occurring toxigenic fungi moulds in banana plantations, and a public health impact from non-rotating crops of cassava, groundnutsб and maize. Finally, we mention our moderate role in surveillance and monitoring of mycotoxins in family smallholder farmers, informal markets, and cooperatives.

Keywords: *Aspergillus*, food safety, fungal pathogens, *Fusarium*, mycotoxins, pathogenicity, Mozambique.

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I. INTRODUCTION

Agriculture, fisheries, forestry, animal farming, aquaculture, aviculture sectors are the basics of the global development process, as they occupy worldwide more than 600 million smallholder family farms [1], representing globally over 85% of all farms, and producing over 80% of the global food [2].

African smallholder farmers with less than 1 ha, produce around 70-80% of food supply at subsistence level, and although it is critical to improve nutrition in rural settings, it is not an easy task neither a successful long-term food security solution. The move toward large-scale agriculture in Sub-Saharan Africa has failed and small-scale agriculture structure prevails maintaining the vicious circle of poverty, illness, and well-being [3].

A vital link exists between nutritional status (i.e., indigence and malnutrition), mental development, suitable water availability, poor health, inadequate education, and socio-economic level. National development, social and economic security, promotes good community health, reduces mortality, but can only be achieved if people are well fed, a basic Human Right.

While many universities have been teaching nutrition and dietetics for more than 100 years (e.g., Syracuse University, USA in 1917, Portuguese speaking S. Paulo University, Brazil in 1939) in Mozambique the first graduate certificate on Nutrition was only awarded in 2012, which reflects the lack of priority, knowledge, significance, and research given to nutrition and public health until recently. Helping countries and communities generating the knowledge that they need for development should be a prime role of assistance. And aid itself is a learning business that continually evolves as lessons of success and failure become clear. Instead, available aid has been used for emergency response, food aid, food security assistance, maternal and child health, and paradoxically did not alter or even worsen the panorama of chronic malnutrition and millions of people are still food insecure [4].

The promotion for the engagement of the poor, and mainly women, in economic growth by increasing their incentives, opportunities and capabilities for employment and entrepreneurship was not achieved [5]. Therefore, the move toward more extensive agriculture in Sub-Saharan Africa has failed, and small-scale farming structure prevails maintaining the vicious circle of poverty, illness, and well-being [6].

While the world currently produces enough and surplus food for its citizens [7], hundreds of millions of people are undernourished with widespread micronutrient deficiencies, mainly in Sub-Saharan Africa, due to conflicts, weather disasters, and lack of technology or productivity [8]. Despite many top United Nations Programs, Global Summits, Agendas, Conferences, Relief Foundations, Charity Groups, Alliances, Meetings, and numerous other initiatives, the Millennium Development Goals were not yet achieved and hunger, malnutrition, food hazards and risks still persist. Further to the need to reinforce agriculture and rural infrastructure development supporting smallholder farmers, it is important to prioritize the transformation of food systems to ensure access to affordable and healthy diets for all, produced in a sustainable manner. However, faster economic growth and expansion of exports in Sub-Saharan Africa will depend on many factors, including efficient, modern standards systems and removal of technical barriers to trade. International standards (e.g., ISO 22000) and technical regulations are usually required for global trade demands facilities and know-how. This is not possible or achievable by small and even medium African enterprises.

The exposure to toxins is unavoidable even in developed countries. Risk assessment is normally difficult with single entities, but when considering multiple mycotoxins and their metabolites, in various food matrices, the nature and magnitude of health risks to humans is very complex to evaluate. A further complication in the risk assessment is the need for research on multiple mycotoxins and metabolites to be monitored on several food and feed matrices and no single entities [9]. These particular kinds of toxins are toxic secondary metabolites of fungi filamentous (hypha) cells, which protect them from insects, bacteria and other fungi [10], [11]. Over 400 toxic metabolites are produced by more than 100 fungi species [12]. Nearly all mycotoxins are thermally resistant and cannot be simply degraded by normal heat treatment methods during food processing or household cooking methods [13], [14].

In fact, if agricultural crops are contaminated by fungi, these substances can enter the food and feed chains. The ingestion of products contaminated by mycotoxins by both people and animals can be harmful to their health and the economy. Furthermore, liver carcinoma that has been related to mycotoxin exposure [15], may arise by multiple predisposing factors including excess weight, alcohol intake, lack of physical activity, chronic viral hepatitis, gut microbiota composition, or smoking [16], [17]. Still, there are not many epidemiological or clinical studies therefore no data to develop adequate public health strategies [13], [14]. Even the World Health Organization (WHO) has based assessment of population-level burden of mycotoxins intake on recent but only empirical data, by indirectly evaluating a population's probable external exposure with no firm biomarker seroprevalence and biological fluids data [18].

As exhaustively reviewed, mycotoxins in food and feed affect renewal or replacement of gut epithelium cells, causing inflammation, reducing self-repair mechanisms, and crossing gut wall into the bloodstream [19]. Chronic viral hepatitis infection is the principal cause of liver disease-related deaths, traceable to common cirrhosis and hepatic carcinoma in Sub-Saharan Africa (more than 26,000 deaths a year) and particularly in Mozambique [20]. Concurrent liver high exposure to food adulterated with aflatoxin-B1, increases the serious hazards inducers of notable human liver diseases, lymphomas, and mortality [21].

Concerns about food security and safety have gained momentum during the last decades, as exports of products from developing countries to advanced economy markets have raised sharply. As we have previously considered, to adequately address food safety issues, consideration must be associated with many other factors such as public health, water supply, sanitation, nutrition, food production, distribution, storage, and marketing issues [22]. In Africa, and since prehistoric times, the majority of the population utilize local medicinal plant products, herbs, and mushrooms sold at local markets to address their primary healthcare needs and wellness [23]. Healing plants and fungi produce thousands of bioactive beneficial compounds, some useful and utilized as antimicrobial therapeutics or growth promoters while others implicated in health disorders and even as biochemical weapons [24]. The rich diversity of plant secondary metabolites may be the source of new antibiotics and plants from tropical and subtropical regions however, besides the valuable bioactive phytochemical compounds, plants may produce other metabolites which may have pharmacological and poisonous effects in human health [25].

With unknown or approved treatment, in humans or animals, mycotoxicoses or poisoning by these natural contaminants depend on wide and multiple factors, including genetic, individual, nutritional, and environmental [26]. Optimal conditions for the biosynthesis of most mycotoxins are moist climate and temperatures ranging from 20 °C to 30°C as those prevailing in Sub-Saharan Africa [27]. Maize grain, cassava and groundnut are major staple foods in this region, providing energy, crude proteins, and income, but stable climate conditions, poor agricultural practices and substandard postharvest food handling facilities support the contamination with filamentous fungi responsible for production of mycotoxin and their toxic group of secondary metabolites [28].

It is well established that in Africa most populations are continuously and seriously exposed to mycotoxins through contaminated foods and beverages [22], [29]. Most available data on the prevalence of mycotoxins in Africa is from feeds [30]. The occurrence of aflatoxins in some Mozambican foodstuff has been reviewed since the 70’s [31] and its adverse consequences well characterized [32], [33]. A recent African Continental Free Trade Area (AfCFTA), was established from 1st January 2021, aiming at overcoming the very little international trade in staple foods among the majority African countries as they are mostly marketed domestically. The predominant (ca. 80%) informal African markets, although not necessarily dangerous lack the ability to impose food safety directives, presenting possible hazards but not necessarily immediate risks. In Mozambique, there is still an accrued significant problem since the northern population prefers grey cassava as staple food, which is indeed contaminated by grey-coloured *Aspergillus*. This *Aspergillus* genus comprises 344 species, *Aspergillus flavus* and *Aspergillus parasiticus* [34], being the most common microfungi in Mozambique [35], [36].

Present authors (TF, JF, IC) have established a public-private partnership internationally accredited laboratory in Nampula, north of Mozambique, with the main objective of controlling mycotoxins levels namely in groundnuts destined for export. The task of establishing certified testing and techniques with ISO17025 standards is onerous and troublesome yet paramount where supply of water and energy suffer regular collapses. Equipment must be imported; people must be trained, and international monitoring is a regular task.

The everlasting malaria epidemics and the current coronavirus disease (COVID-19) pandemic public health crisis cannot hide the constant and neglected major phenomenon of food and beverage contamination with mycotoxins, hindering food safety and healthy long-lifespan of African people and communities [37]. Strategies for preparing the fields for planting crops, their collection, warehousing and processing and spreading the knowledge of existing guidelines and regulations, although being the subject of numerous articles in developed countries, are difficult to be implemented in Africa [38].

During the last five decades many studies have been reported on the type of microfungi and mycotoxin contamination, on the methods of effective monitoring and decontamination, on the search for alternative uses of contaminated products and on the role to be played by local research laboratories [39]. New preventive approaches in controlling fungi and mycotoxins that can jeopardize food safety and consumer health have been widely investigated [40]. In Mozambique, mycotoxin contamination of cassava, groundnut and maize is common, consumers being chronically and alarmingly exposed to mycotoxins with severe health consequences and well-being [33]. Surprisingly, Mozambique exports (126M metric ton in 2020) sesame (*Sesamum indicum*) seeds of so-said premium quality yet there is no reliable data available on contamination of this commodity.

In this review we avoid the already deeply debated issues on relevant producing moulds, outlining a brief overview on the main mycotoxins in Mozambique and the sparse existing strategies and research in one of the poorest countries in the world in a way to safeguard the health of over 30 million people.

II. THE ECONOMIC BURDEN ASSOCIATED TO MYCOTOXICOSIS

A. *Fungal Infections*

Fungal tropical diseases have been constantly neglected over the years, and frequently underestimated as causes of disease and death worldwide [41]. Health practitioners, and even national and global health organizations, under-recognize the true burden of fungal diseases [42], yet its prevalence is increasing due to population ageing and additional compromised immune systems.

However, importantly, fungal infections, are responsible for 150 deaths per hour worldwide, silently resulting in several times more deaths than malaria or AIDS, some 1.7 million deaths per year worldwide, affecting over one-seventh of the world population. Notwithstanding, the influence of mycotoxicoses on general well-being remains ignored by public health services and governing authorities, who have undervalued them mainly in relation to malaria and HIV/AIDS [41], [43]. This underestimation, or even a blind eye, by health authorities, and also sometimes a misleading diagnosis between tuberculosis and fungal infection, is surely related with priorities given to lines of international funding available for research and development.

Other fungal infections occurring presently in India and Brazil [44], yet unidentified in Mozambique but in need of investigation, are the fungal opportunistic, uncommon but serious infection, from *Rhizopus oryzae*, that can be devastating in cases of Covid-19-associated pulmonary mucormycosis, as it is usually found in nature associated with decomposing organic vegetable wastes in soils and dung [45]; and from *Histoplasma capsulatum var. duboisii*, that causes histoplasmosis, which is a systemic mycosis affecting mainly skin and subcutaneous tissues and more rarely lungs, highly endemic in certain regions and increased with the HIV/AIDS epidemic [46]. On one side, this example provides evidence of the economic impact of mycotoxicosis on food production and, on the other side, also indicates the necessity to make available even in developing regions more effective treatments that could contribute to adequately counteract these burdening conditions for local economy and well-being.

B. *Fusarium Oxysporum: The Golden Catch*

Fusarium oxysporum is a cross-kingdom fungal pathogen that infects plants and humans, classified as *Hypocreales*, an order of fungi within the division of Ascomycota, class *Sordariomycetes*, containing 6 families, 137 genera, and 1411 species [47]. All *Fusarium* species synthesize toxic secondary metabolites, mycotoxins [48]. Infectious keratitis caused by filamentous fungal pathogens within the genus *Fusarium*, *Aspergillus*, *Curvularia*, are the major causes of corneal infections in the developing world [49], [50].

Originating in Taiwan in the 1980s, a new strain of the highly invasive banana *Fusarium oxysporum* f. sp. *cubense* began to spread reaching China, Indonesia, Malaysia and the Philippines and even northern Australia, killing millions of banana plants in Asia. In Mozambique, banana plantation started in 2008 with some 300 hectares with Norway´s Aid in Namialo, Nampula, while planned for 6,000 hectares, and at its peak was exporting 1,400 tons of bananas a day. However, in 2013 the plantation was found to be affected by Panama disease (Fig. 1), which devastated the plantation by affecting approximately half a million plants within 3 years. Soon it was closed but anyway endangered the whole national banana industry since over the next few years it could probably spread across Mozambique.

Fig. 1. Banana plantation infested with *Fusarium* in Namialo, Mozambique.

Panama disease, reported as early as 1890 in this Central American country, is caused by the fungus *Fusarium oxysporum*, which lives in soil and enters the plant through the root, blocking the flow of water and nutrients. *Fusarium* wilt on Cavendish plants was observed in the province of Natal of South Africa, as early as 1940 [51].

Phytotoxic metabolites closely related to banana fungus *Fusarium* were identified [52] and can be useful for the prediction of its virulence on banana plants [53]. The fungus lasts in soil for decades and cannot be managed with chemical fungicides. It is easily transmitted in dirt on shoes and car tyres and is probably impossible to control. A new Taiwanese Cavendish strain, known as Formosana, resistant to the fungus, is presently cultivated in the same fields, still as a monoculture, aiming to revive the plantation [54]. Meanwhile, several fungal endophytes were identified with antagonistic activity against *F. oxysporum* f. sp. *cubense* TR4 from isolated weeds growing in Cavendish banana farms [55]. Recent data shows that various new cultivars possess high levels of resistance to Foc TR4 although these studies need replication in different environment and in commercial farms [56].

C. *Fusarium Binding with Gold Particles*

Fungi are an irreplaceable group of microorganisms, despite their value on industry, bio- and nanotechnology are still understudied. The behaviour of various filamentous fungi in the presence of many metals has been widely reported. Fungi have a high level of resistance to all metals and have evolved often complex regulatory networks, uptake and detoxification systems for essential metals.

It is well known the role of bacteria on the biogeochemical cycle of metals, but less information exists on filamentous fungi [57]. Metals are critical for fungal growth phase and metabolism, with a role on infection systems, as they act as cofactors in a lot of enzymes, such as superoxide dismutases, metalloproteases and melanin-producing laccases. *Fusarium oxysporum*, an ascomycete fungus is known to increase the production of radical oxygen species|/radical nitrogen species (ROS/RNS) upon exposure to toxins, facilitating the oxidative dissolution of gold, increasing its gold oxidation potential through the action of superoxide that is a common microbial metabolic by-product [58]. During plant-pathogen connection, plants are known to yield ROS on an oxidative burst as a defense mechanism against pathogens. For a successful infection, these pathogens use different strategies to elude the defenses and secrete enzymes and proteins as reducing agents to counteract that defense [59]. Although the cellular mechanism leading to the biosynthesis of gold nanoparticles is not yet fully understood, *F. oxysporum* f. sp. *cubense* could be an excellent source for the biogenic synthesis of metal nanoparticles extracellularly and intracellularly [60].

It is well established that fungi (e.g., *Trichoderma*), rhizosphere bacteria, and other microorganisms are essential to weathering, the chemical and physical breakdown of rock or minerals. Gold is often considered the most malleable and inert of all metals, nevertheless, the soil-borne fungal pathogen *F. oxysporum* is an essential organism on mineral disintegration and can start gold oxidation under Earth's crust conditions. This fungal is, therefore, associated with the genesis and arrangement of dissolved gold species (Au3+) and gold nanoparticles (AuNPs) [61]. *F. oxysporum* soilborne fungal pathogens have also been previously identified in Colombia and Bolivia as the killing agents of coca plants [62]. Extensive systematic reviews have been published on the role and economic burden of *F. oxysporum* and their formae speciales and races [63], [64]. Over 150 special forms have been described within the *F. oxysporum* species complex based on their ability to cause disease on different host plants. Plant pathogenic fungus *F. oxysporum* f. sp. *cubense* JT1 (FocJT1) has been reported to secrete various virulence factors, such as cell wall-degrading enzymes, effectors, and mycotoxins, that potentially play important roles in fungal pathogenicity for its ability to reduce gold ions producing nanoscale particles of solid gold [65], [66].

These findings on the role of *F. oxysporum* f. sp. *cubense* race TR4 could help miners find the next generation of underground gold deposits since the fungus can be used in conjunction with other exploratory techniques to target prospective areas in a way less impactful and more cost-effective than drilling.

The interaction between fungi, other microbiota components, water, soil, and minerals, expected to influence gold distribution in the area, and the ongoing redox process under Earth surface conditions, reveals the key abiotic factor of gold rather than just an inactive element in sustaining fungal ecosystems in the gold-bearing environment (Fig. 2) [61].

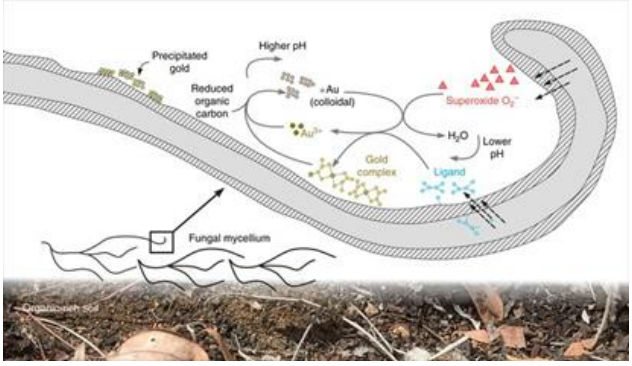

Fig. 2. The conceptual model for mycological gold (Au) redox transformation under Earth surface conditions. Dashed arrows indicate superoxides and ligands that are produced intracellularly (Adapted from [61]).

Microorganisms, namely fungi, offer a distinct environment for nanoparticle synthesis, which may be used in medicine, agriculture, environment, therapeutics approaches and biosensors [67], [68]. The primary oxidant from fungal mycelium hyphae consists of enzyme superoxide dismutase, which breaks down colloidal gold to gold ions with expected support of composite particle positive protons [69],[70].

III. HEALTH HAZARDS OF FUNGAL FOOD CONTAMINATION

A. *Toxigenic Fungi and Mycotoxins*

The prospects of eliminating aflatoxin in the near future at the household level and in trade are not promising. Factors that contribute to mycotoxins (e.g., aflatoxins, fumonisins, zearalenone, deoxynivalenol, ochratoxins) contamination of foods and feeds are mainly related to ecosystem circumstances, namely humidity and temperatures and monotonous diet, which may support fungal upsurge and toxin genesis.

As already seen, certain crops that are common in the diets of poor populations worldwide, such as corn and cassava, can be compromised due to naturally occurring foodborne toxins. When humans rely almost single-handedly on a single staple crop, not only are there nutritional deficiencies, but any toxins that naturally occur in that crop will accumulate in the body, causing harmful health effects. As a result, foodborne toxins are often consumed unknowingly at varying concentrations, and children are often at highest risk from adverse health effects [71].

Despite the reported high dietary levels of mycotoxins, legislation for their control is absent in most countries in the region, and when existing, regulations on contamination are not effective in subsistence farming communities. In central Mozambique there are 15 processors and 5 cooperatives delivering 3.7 million kg of milk products per year, and there is no vigilance, therefore significant danger on the metabolite aflatoxin M1 in milk is carried over to consumers [72]. Other parts of the country rely on imports of dairy products [73].

Actually, most fungi do not produce mycotoxins. Micro fungi moulds of the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Claviceps* and *Neurospora*, are the most common producers of as mycotoxins developed during production, harvesting, or storage of crops and have a adverse effects on the human and animal health [72]. Large number of mycotoxins have been identified, but only about 20 of them manifest noteworthy food safety challenges, as they enter the food and feed supply chain. Although many fungal metabolites have been identified, only a fraction have been evaluated in international risk assessments [74].

Mycotoxins can be classified in four primary toxicity types: acute, chronic, mutagenic, teratogenic. Mycotoxins are recognized as the main determinants mutagenic and carcinogenic elements [75]. These toxins are found worldwide as natural contaminants in products of plant origin, such as cereals grains, cassava, nuts, oilseeds, fruits, dried fruits, vegetables, beans, herbs, and spices (Fig. 3). Fruit- and vegetable-based beverages such as wine, beer, tomato, and apple juices are prone to contamination with ochratoxin A, patulin, deoxynivalenol, and aflatoxin M1, even in Europe.

Fig. 3. Food and feed usually associated with mycotoxins.

Some foods and feeds are often contaminated by numerous mycotoxins, but most studies have focused on the co-occurrence and toxicology of dual mycotoxins, namely aflatoxins in hot dry weather and fumonisin in hot wet season. Toxins can remain in the organism after the fungus has been removed [76]. Fungi mycotoxins, contrary to bacterial toxins, are non-protein secondary metabolites and therefore are not noticeable by the human and animal immune system. Even in Europe the evidence does not allow the derivation of a chronic health-based guidance value for cyanide and thus chronic risks could have not been assessed [77]. In contrast to the infectious diseases, mycotoxins have been neglected in most developing countries despite their chronic effects on human being. No data is available on mycotoxin contamination of Mozambican commodities although our own unpublished surveys, conducted on samples from a cooperative with some 120,000 small producers, showed levels of contamination of 100% of dried “grey” cassava (called *karakata*), a traditional and preferred staple food in the northern region. It should be enhanced that our studies with cashew nuts shows that these in general are not contaminated with mycotoxins, easing exports.

In Mozambique there is a commercial beer plant made from fresh cassava delivered by 6,000 local producer, and levels of some mycotoxins are routinely evaluated in the final product and reported as very low. Although the data on impact of mycotoxins on intestinal functions is scarce, gastrointestinal cells and the microbiota are the first to be in contact, and at higher levels than other tissues increasing gut mucus secretion. In addition, mycotoxins specifically target the biological functions of scaffold proteins and aggregated proteins, which are predominant in gut epithelium [78]. Cells and microorganisms in the gut microbiota live in symbiosis and any disturbance may affect homeostasis causing inflammation, immune dysregulation, disturbing autoimmunity and metabolisms, and spawn neurodegeneration [79]. Mycotoxins affect vulnerable structures in the intestines, in particular the gut barrier function and bacterial translocation, impairing the epithelial lining cells and the sealing tight junction proteins with liberation of antimicrobial peptides. This could result in paracellular transport of luminal antigens and pathogens and an additional activation of the innate immune system generating prolonged inflammatory responses (Fig. 4) [80]. The progress in the characterization of the intestinal toxicity of mycotoxins is quite recent. Mycotoxins, with chemical structures as diverse as aflatoxins, ochratoxin, and deoxynivalenol (DON), have been shown to impair intestinal permeability in various human and animal species. These studies will only be complete when further data on the role of microbiota is clear in this domain [81].

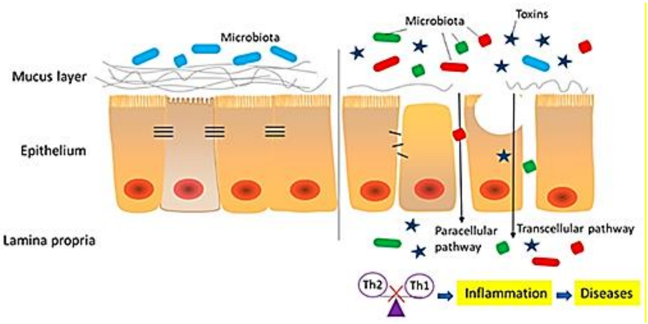

Fig. 4. Mycotoxin effects on the intestinal barrier [80].

The "natural" and unavoidable contamination with mycotoxins is a health hazard worldwide. DON, is one of the most widespread mycotoxins in Europe, mainly produced by *Fusarium graminearum* and *F. culmorum*. It frequently contaminates cereals and cereal products with half the samples analyzed being contaminated in Europe [82], [83]. The yeast *Saccharomyces boulardii* is a unique, non-bacterial microorganism classified as a probiotic agent and has been used successfully in the treatment or prevention of several gastrointestinal diseases through production of elements deterring pathogens, impediment of pathogen attachment, and hampering the action of toxins (Fig. 5) [84].

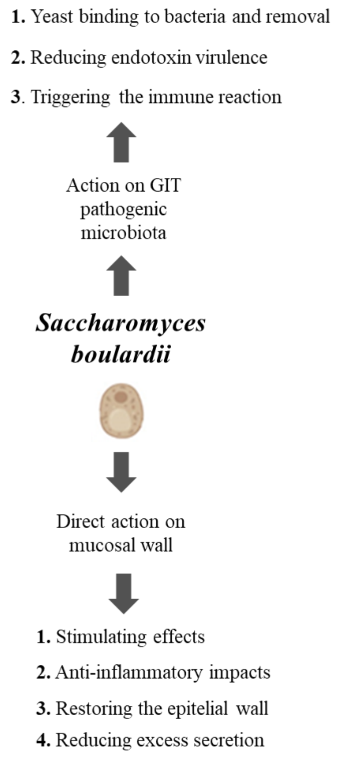

Fig. 5. *Saccharomyces boulardii* may be helpful in mycotoxins infections.

B. *Toxigenic Fungi and Mycotoxins*

Mozambique food production is not diversified, and rural people eat monotonous diets. Maize in the south and center of the country and cassava in the north are the staple foodstuffs (Table I).

TABLE I: MAIN FOOD COMMODITIES CONSUMPTION IN MOZAMBIQUE [85]

Commodity	Quantity consumed (Kg/person/year)	Daily caloric intake (kcal/person/day)	Share of total caloric intake (%)
Maize	58	462	22%
Cassava	247	740	36%
Wheat	20	147	7%
Rice	15	145	7%
Others	87	587	28%
Total	427	2082	100%

C. *Cassava*

Cassava (*Manihot esculenta*) is the staple food and source of nourishment for more than one billion people worldwide. Cassava is the most important starchy staple food in Mozambique (some 9 Mt; 6,000 kg/ha in 2019), along with maize, rice, sweet potato, beans and millet. Its production is mainly concentrated in four provinces in central and northern Mozambique (Zambezia, Nampula, Cabo Delgado and Niassa) (Fig. 6). Several processing units of cassava were established with a capacity of some 700 Kg /day in order to stimulate cooperative work and consumption of this basic food.

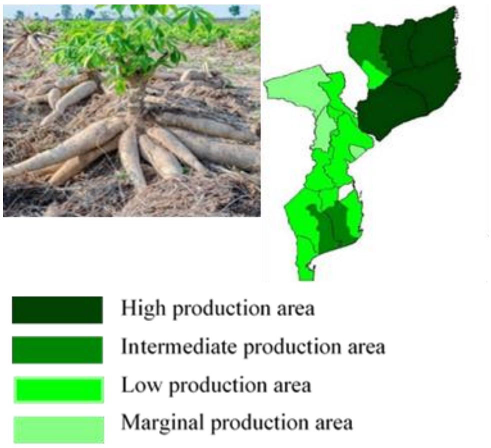

Fig. 6. Cassava, a staple food in Mozambique denoted in dark green (IIAM, 2007, cited by [86]).

Cassava leaves are picked and consumed regularly as local spinach. Some 8 to 24 months after planting cuttings by hand, roots of cassava are harvested and undergo on-farm processing as it can degrade immediately after picking and cannot be stored for very long. Depending on the region, cassava is consumed fresh or dry and, when it is not consumed fresh, cassava is processed using simple tools or through mechanical equipment in specialized industries.

The expanding cassava markets in Mozambique are centered on fresh cassava, cassava flour, cassava-based starches, biofuels, and feeds. In the past, a small outbreak of paralysis and poisoning in a cassava-dominated rural area of Mozambique revealed that walking disability (locally called *mantakassa*), was the well-known neurological disorder konzo due to insufficient processing of the bitter cassava roots. More recently, tropical myeloneuropathies, cognitive impairments, and neurodevelopmental delays have been reported among young children above the age of three due to dietary cyanogen exposure [87]. Most processing in Mozambique is homemade to eliminate cyanogenic glycosides, such as linamarin and amygdalin, in pest resistant bitter varieties. Strangely, although there are several methods to quantify glycosyl cyanides, there are no validated methods (by FDA or EFSA) available for the quantification of cyanogenic glycosides in food items [88]. There is a lack of awareness and sufficient information on the risk associated with consequent of mycotoxin contamination on wellbeing of human, animal health and the economy in Mozambique.

Consumption of cassava contaminated with mycotoxins is inevitable, hence the need for adequate field management practices while proper regulation is not in place. Some authors suggest inclusion of incorporating probiotics, and hydrated sodium calcium alumino-silicates into the diet, but this is not practical [89], [91]. We have tested the effectiveness of Mozambican diatomaceous earth and bentonite clay in reducing the toxic effects of aflatoxin B1 in chicks. Diatomaceous earth was not effective in reducing toxic effects while bentonite clay showed promising data. Low levels of productivity for cassava compared to elsewhere and poor transportation are the main barriers to the development of cassava processing industry [92].

Because the quality of fresh cassava is quickly deteriorated in 3 days, farmers keep some fresh cassava for self-consumption and sell the rest immediately in village markets. A large quantity, up to 40,000 tons of raw cassava sourced annually from over 1,500 small farms, is sold to large beer factories, specialized in 70% cassava-30% barley commercial beer that organize the collection of roots or their cakes from local farms and transport to industrial plant. Traditional beers frequently contain mycotoxins and even industrial beer in Southern Africa is contaminated.

A decrease of <20% aflatoxins can be achieved during the industrial production while >50% of deoxynivalenol and higher levels of fumonisin B1 can be recovered in the finished processed beer [93]. While the exposure of cassava consumers in Nigeria, the world’s largest cassava producer, to regulated (by EFSA) mycotoxins was estimated to be minimal [94], in Mozambique a proper assessment of the risk of exposure of humans and animals to high concentrations of mycotoxins of known toxicity has not been conducted on a systematic way.

D. *Maize*

Maize (*Zea mays*) production has been very volatile, with generally poor quality, being predominantly of white maize type with lower content on β-carotene [95]. Yellow maize grain and transgenic species from multinationals are imported from South Africa and not common and relatively few grain processing services are available [96], [97]. While maize production in Mozambique has been reported as 1,9 million tons for 2020/21, there is heavy informal cross border trade across the region rendering production estimates redundant although maize supplies likely meet national food consumption needs [98].

Maize is the staple diet in the region with maize flour mixed with salt into a paste known as xima (nshima) mealie meal in Southern Africa. Maize contamination with aflatoxin, mainly Fusarium, is a long-standing problem [99]–[101]. Fumonisin B1 concentrations in maize in Mozambique (92% incidence, median = 869 μg/kg) are quite high [102]. In neighbour South Africa, transgenic (Bt) maize showed some 40% less fumonisin than the traditional maize [103]. Significantly lower levels of fumonisin have been demonstrated in *Bacillus thuringiensis* (Bt) maize, through reduction of insect pest damage and subsequent fungal infection [104],[105].

For more than three decades now, the problem of aflatoxin in maize has been researched in Africa [106]. Many different solutions to mitigate aflatoxin contamination in maize and nuts have been developed and are well reviewed [107],[108].

E. *Peanuts*

National markets do not restrict the sale of aflatoxin-contaminated peanuts and exports of peanut oil and its fractions, not chemically modified, to Switzerland were up to $80M a year. Aflatoxins have been determined in Maputo, south of the country, and levels were found acceptable at supermarkets, with average 2.71 μg/kg raw peanut, but mycotoxins continue to be a problem in both formal and informal trade [109], [110]. There is a frequent misconception based on solubility considerations and developed market surveys that aflatoxins do not occur in peanut oil, but they can contain high contamination levels [111]. In fact, there is a general poor awareness about aflatoxins, let alone the dissemination of appropriate control measures and governmental extension services need to be trained to include this on their information to communities. We have been monitoring mycotoxins regularly under contract with a cooperative (IKURU) with 20.000 small farmers (40% women, 60% men) with a production area of over 300.000 ha cultivation of peanuts and sesame in Nampula, Mozambique [112]. Data in Nampula hotspot is quite different with most peanut samples contaminated up to 6mg/Kg raw material despite the introduction of some mitigation techniques [112].

IV. LABORATORY PROCEDURES FOR MYCOTOXIN SURVEILLANCE IN MOZAMBIQUE: STATE-OF-THE-ART AND MYCOTOXIN OCCURRENCE

A. *Sampling*

Mycotoxin levels are very uneven varying among individual grains and products in a contaminated lot, with great variation from sample to sample, rendering the process of obtaining a representative sample quite complex. We took special training in the intricate area of sampling for mycotoxins in a Southern Africa Regional Meeting debating adequate sample size, location of test sample, identification, labelling and transportation.

Although excellent analytical methods are available, it is difficult to estimate accurately and precisely the mycotoxin concentration in a large bulk lot because of the large variability associated with the overall mycotoxin test procedure, and nearly 90% of the error associated with mycotoxin testing can be attributed to sampling. Mycotoxins do not distribute evenly and usually the unit measurement for mycotoxins is 1 ppb which is 1 part in 1.000.000.000, 1 grain of sand in 22 kg, 1 maize plant in 40.000 acres of maize plantation, 1 grain of maize in 3.5 railcars. We regularly survey mycotoxin contents of market-bought grain, peanut, and cassava samples in order to assess the threat these mycotoxins might represent to the population [113]. The present research survey was carried out in seven districts of Nampula province, with semi-arid and dry sub-humid climate and with an average of annual precipitation between 800-1,200 mm. The annual average temperature varies from 20 °C to 25 °C and is at an altitude of 200-500 m.

In this instance, a total of 120 samples (ca. 200 g each) were collected through donations and/or purchases from various local smallholders and farmers' markets. Each sample was ground into a fine powder (final fineness < 40 µm) and stored at 4 °C in air-tight containers for few weeks until further processing. Testing for mycotoxins generally consisted of three steps: (1) several small samples were taken at random from the lot and composed into one larger "lot sample", (2) the entire lot sample was ground to a fine particle size and a representative subsample, the "analytical sample", removed for analysis, and (3) the mycotoxins extracted from the analytical sample and finally quantified [114].

B. Sampling

There is a comprehensive range of mycotoxin test kits to detect mycotoxins (aflatoxins, DON, fumonisin, ochratoxin, T-2/HT-2, and zearalenone), which provide results in minutes, while requiring minimal training and equipment. The supply of pure standards for different mycotoxins may be the only obstacle as they need importation which may impair planning. Mycotoxin levels were measured using fluorometer Series-4EX (VICAM, USA). The method is fast, simple, safe, and highly accurate and validated by the Association of Official Analytical Chemists (AOAC) applied for aflatoxin (B1, B2, G1, G2) and fumonisin using the following procedures: (1) Sample extraction: 25 g of samples weighed with 5 g NaCl and placed in blender jar. Add to jar 125 mL 70% methanol: 30% water (v/v). Blender jar was covered and blend at high speed for 2 min. Once removed the cover from the jar and extract was poured into fluted filter paper. Filtrate was collected in a clean vessel; (2) Extract dilution: 15.0 mL filtered extract was poured into a clean vessel. Extract was diluted with 30 mL distilled water and well mixed. Diluted extract was filtered through a glass microfiber glass filter into glass syringe barrel using marking on barrel to measure 15 mL; (3) *Column chromatography*: 15 mL of filtered extract was passed completely through the AflaTest column at a rate of 1-2 drops/second (15 mL = 1.0 g sample equivalent). The column was washed with 10 mL of distilled water at a rate of 1-2 drops/second. Again, the column washed with 10 mL of distilled water at a rate of 1-2 drops/second, until air comes through column.

TABLE II: AVERAGE LEVELS OF TOTAL AFLATOXIN AND FUMONISIN B1 IN DIFFERENT PRODUCTS (1 PPB = 0.001 MG/KG)

Toxin	Product	Average (ppb)	SE
Aflatoxin	Maize	17.51	11.58
	Maize flour	41.49	25.24
	Feed compound	3.10	2.10
Fumonisin	Feed compound	2.35	0.85

TABLE III: AVERAGE LEVELS OF TOTAL AFLATOXIN IN PEANUTS IN FOUR NAMPULA DISTRICTS IN 2020/21 (1 PPB = 0.001 MG/KG)

Average levels of aflatoxin in peanuts in four Nampula districts in 2020/21

Localities	Average (ppb)	SE
Erati	19.69	9.81
Meconta	16.50	9.73
Mogovolas	21.23	10.57
Chiuri	30.83	10.51

AflaTest column eluted with 1.0 mL high performance liquid chromatography (HPLC) grade methanol at a rate of 1-2 drops/second and collected all of the sample eluate (1 mL) in a glass cuvette. 1.0 mL of freshly made AflaTest Developer (used to give colour) added to the eluate in the cuvette. After well mixed fluorescence was measured in a calibrated fluorometer. Aflatoxin concentration was read after 1 min [115].

C. Occurrence of Aflatoxin in Foodstuffs

Present data demonstrates contamination of mycotoxins per kg of fresh food sample and not maximum tolerable daily intake per kg/bodyweight/per day (Table II).

TABLE IV: TOTAL AFLATOXINS LEVELS IN PEANUT SAMPLES

Aflatoxin determined in several peanut batches in 2019/20 (1 PPB = 0.001 mg/kg).

Districts	Localities	Average(ppb)	SE
Erati	Nacole	87.1	0.01
	Mucueger	1.0	0.10
	Chacas	56.6	19.27
	Murima	1.0	0.14
Meconta	Katipa	2.8	0.01
	Teterrene	90.4	37.59
	Nacoma	1.0	0.11

Our analysis reveals that nearly one sample of maize out of four was contaminated with very high levels of mycotoxins. The presence of possible "masked mycotoxins", i.e., mycotoxins biologically modified by the plant thus having modified toxicities and analytical detectability, and a possible combination of different mycotoxins with possible synergistic toxic effects, modified toxicities, and analytical detectability, were not the subject of investigation.

Proper post-harvest treatment by smallholder farmers is essential practice to reduce the risk of aflatoxin contamination, the main one adopted with success has been the open-air drying (Fig. 7) together with adequate storage. The use of resistant varieties is slowly introduced but still with unknown impact while other techniques have been reviewed but with no sound application in the field [116].

Fig. 7. Three different ways of open-air groundnut drying in Mozambique.

The application of non-toxin producing isolates of A. flavus to replace this major aflatoxin producer is still an experimental practical method claimed to effectively limit aflatoxin contamination in crops from farm to dish [117]–[119]. After more than a decade developing and field-testing this innovative, simple, but commercial way to control aflatoxin in peanut and maize, and despite many uncertainties, in 2019 it has been approved by the authorities in Mozambique.

V. Discussion

Most developing countries follow the *Codex Alimentarius* Commission, supported by FAO and WHO since 1963, which aims to ease and promote world commerce and safeguard the health of consumers through the development of international standards for foods and feeds and their maximum limits (standards) for contaminants. Comprehensive databases were created in Europe but even in this developed scenario the existing gaps are huge and an obstacle for the harmonization of standards due to the absence of common statistical and study designs [120]. Indeed, proposed standards for different mycotoxins in different substrates are permanently under discussion with new data available but strongly dependent on sampling procedures [121] and the risk of mycotoxin contamination is underestimated in the animal industry [122].

In most African countries, maximum mycotoxins tolerable intake levels follow outdated *Codex Alimentarius* guidelines which moreover has no legitimacy in rural malnourished populations under subsistence farming. There is an absence of trans-disciplinary nutrition-agriculture research programs in order that a holistic approach can be implemented, to recognize the integration between food production and consumption. Measures to prevent and reduce the contamination have been gradually introduced across Africa, but even in developed countries maximum tolerable limits have been regularly reviewed due to recent climate changes. Only about 15 countries in Africa had aflatoxin regulations covering 60% of the inhabitants of Africa, but only South Africa expanded to other mycotoxins [123].

The main overall objective of authorities has been the development of Codes of Practice in which principles in food hygiene and advice about practical measures to control, prevent and reduce mycotoxins during cultivation, storage and processing are assembled [124]. Notwithstanding the humanitarian impulse of many of organized international institutions, philanthropic foundations, public and private organizations, agencies, and voluntary organizations, they have polarized this control as they are heavily subsidized for research with commercial interests. These initiatives may have a restricted value since Africa cannot be once more dependent on new commercial products to effectively control the ill-health of the tropic's questionable utility. Despite reasons for optimism, the promise of authorities to invest in R&D remains unfulfilled and laboratories, equipment and skilled staff are scarce in Mozambique and most Sub-Saharan countries. The testing of new biocontrol initiatives which are commercially based and backed up by few studies supported by international and aid organizations, for-profit programs and products, are promising but not suitable for different purposes and may not be successful on the long run in African settings [125]. The use of registered commercial atoxigenic isolates of *Aspergillus flavus* may require new guidelines for manufacture, specification and regulation and is not universally appropriate for all pest management situations [126].

Numerous secondary metabolites produced by *Trichoderma* species could directly also inhibit the growth of several plant pathogens. *Trichoderma*-based biocontrol mechanisms rely on mycoparasitism, production of antibiotic and/or hydrolytic enzymes, competition for nutrients, as well as induced plant resistance [127]. Colliding population waves of trophically identical but fitness different species can interpenetrate through each other. However, growth of toxigenic A. flavus strains is not inhibited in biocontrol-treated grain during post harvested incubation period. Indeed, pre-harvest biocontrol applications does not replace the need for better post-harvest practices that reduce the drying time between harvest and storage [128].

The resilience of pre-harvest and post-harvest of non-toxigenic strains of *A. flavus* for control of aflatoxin contamination of maize cultivars was investigated and it was shown that water availability, temperature and other environmental factors influence both type of strains, being less effective at the dent stage and in non-genetically modified maize [129], [130]. Ectomycorrhizal fungi through its properties of mineral dissolution and precipitation, organic matter decomposition, plant symbiosis and degradation of silicates, influences the ecosystem biogeochemical cycles whereupon life on earth subsists, therefore the mechanisms that maintain the richness of fungi communities is critically important [131].

Although root endophytic microbiota is more determinant than soil microorganisms, the extent to which root fungi influence the microbial community assembly is not clear as such studies are quite challenging [132]. Therefore, it is still undetermined the real impact of the new biocontrol methods on the microbiota of soils and plant roots [133] and a consensus on best practices in microbiome research is still missing [134], [135].

Microorganism species generally have a particular set of traits that make them well suited to persist in certain environments but not in other ecological communities having different competitive dynamics [136]. Furthermore, competition-colonization trade-offs, i.e., increase in performance in one area being correlated with a decrease in performance in another area, may facilitate coexistence in microorganisms but this subject remains understudied and the application to ectomycorrhizal fungi remains largely unknown [137]. Therefore, the mechanism of action and the inoculum dose necessary of biocontrol agents are still unfamiliar territories in need of investigation.

Africa needs to modernize their laboratory health institutions, improve research training capacity of staff, and promote the importance of public health among countless populations and prepare vast regions for investment and increased productivity [138]. No comparable statistical data is available on mycotoxin contamination in Africa and Mozambican foodstuffs, although our own unpublished surveys, conducted on samples from a cooperative with some 120,000 smallholders peanut farmers, showed levels of contamination of 100% of dried "grey" cassava, a staple food in the northern region.

Not being a cross-border disease, we have focused on national aflatoxins, and fumonisins but not on zearalenone, deoxynivalenol and ochratoxins, although they were occasionally determined, in order to support the role of public health by swiftly detecting food contamination and training new academics.

VI. CONCLUDING REMARKS

While people are more aware of bacterial and viral pathogens, fungi are becoming more successful pathogens, threatening agribusiness, human health and causing 4 times more deaths than malaria. The full cost burden of mycotoxins contamination is unknown. Our surveys and research only provide few elements, enabling us to monitor and refine the curtailment of potential health risk associated with post-harvest losses and mycotoxin levels in smallholder farmers, and help us define adapted local food safety standards.

In the northern region of Mozambique, traditional and preferable use of yeasty and mouldy maize and grey cassava, with the pleasing local acquired taste, increases the danger of harmful effects while processing into various indigenous commodities only slightly reduces their high toxicity. With consumer and intensified regulatory environment, the crop production and protection landscape appear now to be changing rapidly and it is essential to identify a holistic approach to a broad range of issues, including soil conservation, water availability and the need for sustainable and improved pest and disease management practices in order to register any success. This means the need for further measures to simplify, harmonize and minimize the cost of procedures rather than adding new imported and hard to apply regulatory burdens. Only a safe, affordable, healthy, and sustainable food supply will improve health and well-being of rural population.

AUTHOR CONTRIBUTIONS

Conceptualization, T.F.; writing: original draft preparation, T.F.; laboratory analysis: I.C.; writing: review and editing, V.B., J.G., J.F.; visualization: V.B., T.F.; submission: T.F. All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST

All authors declare no conflict of interest.

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