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COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
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Mycotoxins contaminations in red pepper powder produced at households in Addis Ababa

A Thesis Submitted to the Center for Food Science of Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Master of Science in Food Science and nutrition

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June, 2025
Addis ababa, Ethiopia

DECLARATION

I, the undersigned, declare that this thesis entitled "Mycotoxins Contaminations in red pepper powder produced at households in Addis Ababa" is an original work and has never been presented in this or any other university, as well as research center previously. All of the source materials used for this thesis have been fully acknowledged.

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ACKNOWLEDGMENT

Above all, I want to give thanks to the Almighty God. And I would like to express my gratitude to my advisor Dr. Abebe Ayelign who supported me starting from selecting research area and developing my proposal till the end of this research. In addition to being my advisor, Dr. Abebe was a friend who gave me strength when I was feeling down and clarity when I needed it when I was confused. Above all, I will never forget the time he gave me out of his hectic schedule to assist me in gathering red pepper powder samples from households and the valuable discussion I had with him throughout this research. I would also like to thank my co-advisor Dr. Paulos Getachew for his support in all endeavors throughout this research; my grateful thank goes also to Ethiopian Agricultural Authority for the help by giving export permit letters. I would like to thank to Patent Co Laboratory in Hungary for laboratory facilities for the successful completion of this thesis work. Furthermore, I also would like to thank inhabitants of Addis Ababa for their collaborations during sample collection phase.

Last but not least, I am deeply indebted to my friends and lab assistant at Center for Food Science and Nutrition for the good time we had all along.

TABLE OF CONTENTS

Contents

ACKNOWLEDGMENT ..... **I**

LIST OF FIGURES ..... **V**

LIST OF ABBREVIATIONS AND ACRONYMS ..... **VI**

ABSTRACT ..... **VII**

1. INTRODUCTION **1**

1.1 BACKGROUND 1

1.2 STATEMENT OF THE PROBLEM 5

1.3 OBJECTIVES 6

1.3.1 General objective *6*

1.3.2 Specific objectives *6*

1.4. Significance of the study *6*

2. LITERATURE REVIEW **7**

2.1. RED PEPPER 7

2.2. RED PEPPER PRODUCTION 9

2.2.1 PRODUCTION AND CONSUMPTION OF RED PEPPER IN THE WORLD 12

2.2.2 RED PEPPER PRODUCTION IN ETHIOPIA 15

2.3 MYCOTOXINS IN RED PEPPERS 17

2.3. MAIN SOURCES AND MYCOTOXINS 19

2.4. TYPES OF MYCOTOXINS 21

2.4.1 Aflatoxins (B1, B2, G1 and G2) *21*

2.4.2 Ochratoxin A *23*

2.4.3 Diacetoxyscirpenol (DAS) *24*

2.4.4 Enniatins *25*

2.5 IMPACT OF MYCOTOXINS' CONTAMINATION ON HEALTH, TRADE AND ECONOMY IN ETHIOPIA 27

2.6 MYCOTOXINS REGULATION IN ETHIOPIA 31

2.7. RAISING AWARENESS AND ADULTERATION PRACTICES IN ETHIOPIA 32

2.8 MYCOTOXIN MANAGEMENT STRATEGIES 33

2.9 Reduction and Prevention of Mycotoxins 35

3. MATERIALS AND METHODS 39

3.1 Description of the study area 39

3.4 The knowledge and practices survey 41

3.5. Red pepper powder sample collection 41

3.6 Moisture analysis 43

3.7 Mycotoxins analysis 43

3.8 Data analysis 46

3.9. Ethical clearance 47

4 RESULTS AND DISCUSSION 48

4.1 Sociodemographic characteristics of the respondents 48

4.2 Knowledge of the respondants related to mycotoxin contamination 49

4.3 Practices of the respondants related to mycotoxin contamination 50

5. CONCLUSIONS AND RECOMMENDATION 65

5.1 CONCLUSION 65

REFERENCES 67

APPINDIX 1 82

APPENDIX-2 88

APPENDIX_3 94

APPENDEX 4. 97

LIST OF TABLES

Table 1:Top Countries in Pepper Production –in the world Source FAO ……15
Table 2: Major common mycotoxins, their (established/evolving) and toxicities ……26
Table 3: Major common mycotoxins, their (established/evolving) and toxicities …….31
Table 4: Number of red pepper samples collected in each sub city in Addis Ababa for mycotoxin analysis ……42
Table 5: Solvent gradient conditions for the UHPLC-MS/MS…… 45
Table 6: The validation parameters for mycotoxin analysis: limit of detection (LOD) and limit of quantification (LOQ) for each mycotoxinss ……46
Table 7: Sociodemographic information of the respondants, 2024 ……..48
Table 8. Respondants knowledge regarding mycotoxin contamination in red pepper powder, 2024 ……49
Table 9: Practices of respondents on issues related to mycotoxins in red pepper powder ……...51
Table 10: The level of mycotoxins in red pepper powder samples collected from households in three sub cities in Addis Ababa at base line, 2024 ……55
Table 11: The level of mycotoxins in red pepper powder samples collected from households in three sub cities in Addis Ababa after six months storage, 2024 ……58
Table 12: Mycotoxins levels in red pepper powders and moisture content, collected from households in three sub cities in Addis Ababa at base line, 2024 ……62
Table 13: Mycotoxins levels and moisture content in red pepper powders samples collected from households in three sub cites in Addis Ababa after six months storage, 2024 ……...63
Table 14: Mycotoxins levels and moisture content in red pepper powders samples collected from households in three sub cites Addis Ababa before and after six months storage, …….. ……...64

LIST OF FIGURES

Figure 1 dried and ground red pepper……8
Figure 2 critical factors favoring fungal growth and mycotoxins contaminations during the pepper production chain……...11
Figure 3 Regional share global production of fresh and dry pepper…….13
Figure 4 Global consumption of pepper by region……14
Figure 5 Chemical structures of aflatoxin B1, B2, G1 and G2…….23
Figure 6 Chemical structure of ochratoxin A……24
Figure 7 chemical structure of Diacetoxyscirpenol…….24
Figure 8 The chemical structures of beauvericin,enniatin (A,A1,B and B1)……..25
Figure 9: Map of Addis Ababa sub city where the red pepper samples were collected…40
Figure10: process flow diagram for red pepper powder preparation at households……..42

LIST OF ABBREVIATIONS AND ACRONYMS

AFB	Aflatoxin blue
AFG	Aflatoxin green
ANOVA	Analysis of variance,
AOAC	Association of Official Analytical Chemists
BC	Before Christ
CSA	Central Statistical Agency
DAS	Diacetoxyscirpenol
DON	Deoxynivalenol
EC/EU	European commission/Union
EEPA	Ethiopian Export Promotion Agency
EIAR	Ethiopian Institute of Agricultural Research
ENTAG	Ethiopia–Netherlands trade for agricultural growth
EPHI	Ethiopian Public Health Institute
ESA	Ethiopian standards agency
FAOSTAT	Food and Agricultural Organization Statistics database
GBC	Gallbladder cancer
GC	Gas chromatography
HPLC	High-performance liquid chromatography
LC MS\MS	Liquid chromatography mass spectroscopy
MTL	Maximum tolerable limit
ND	Not detected
OTA	Ochratoxin A
PAT	Patulin
SNNPRS	Southern Nations, Nationalities, and People's Regional State
SPSS	Statistical Package for Social Science
TCT	Trichothecense
TFC	Turkish Food Codex
UHPLC ms/ms	ultra high performance liquid chromatography tendem mass spectrometry
UV	Ultraviolet
ZEN	Zearalenon

Abstract

Red pepper *(Capsicum annuum L.)* is the most important world spice crop and the second largest consumed spice throughout the world, after black pepper. Apart from its nutritional importance, red pepper has a huge economic value in many countries including Ethiopia. Despite the role of red pepper in nutrition, income and national economy, it has been susceptible from the risks of mycotoxin contamination. Therefore, this study aimed to evaluate the level of mycotoxin in powder red pepper produced at households in Addis Ababa. Sociodemographic information, knowledge and practices related to mycotoxin in red pepper from 124 households were collected using pretested questionnaires. One hundred twenty-four (124) red pepper powder samples at the time of proessing and after six months storage at house holds in three subcites in Addis Ababa were collected. From the 124 samples collected, 40 composite red pepper powder samples were produced both at baseline and after six months storage and the level of 26 mycotoxins were determined using a validated UHPLC-MS/MS method. Out of 26, ten mycotoxins were detected in the red pepper powder samples both at baseline and after six months storage. The ten mycotoxins were detected in all the samples at concentrations ranging from < LOD to 154 µg/kg and < LOD to 89.7 µg/kg at baseline after six months storage respectively. In this study, 92.5% of the red pepper powder samples at baseline (during processing) and 75% after six months storage was contaminated with AFB1 above the EU limit and Ethiopian standard of 5 µg/kg. Further, the mean levels of AFB1, AFB2, AFG1, and AFG2, Beauvericin, Enniatin B and Enniatin B1 decreased significantly after six months of storage. This might be some spices, like garlic, ginger, and cardamom, have antioxidant and anti-fungal properties, which can potentially inhibit aflatoxin production or degradation. Overall, mycotoxins (such as AFB1, AFB2, AFG1, and AFG2, Ochratoxin A, Beauvericin, Enniatin A1, Enniatin B and Enniatin B1) were widespread contaminants of red pepper powder produced at households in the Addis Ababa intended for human consumption. Therefore, awreness creation among stakeholders about proper preparation methods of red pepper powder and health impacts of mycotoxins is warranted.

Key words: Mycotoxins, Red pepper, Addis Ababa, Household

1. INTRODUCTION

1.1 Background

Red Peppers are one of the most widely used spices in the world (*Abate et al., 2015*), It is grown throughout the country. They are typically planted in tropical and sub-tropical environments (*Berke and Shieh, 2012*).

There are more than 400 different varieties of chillies found all over the world. It is also called hot pepper, sweet pepper, bell pepper (*Idrees et al., 2020*). Its botanical name is *"Capsicum annuum".* Chili is fruit of the plant „*Capsicum annuum" and „Capsicum frutecens*" that come from the genus capsicum belonging to the family of Solanaceace *(madala and nuttaki, 2020).*

Red Pepper (*Capsicum annum L.)* is an exotic crop for Ethiopia and believed to be introduced by the Portuguese in the 17th century and currently considered as the national spice.it is commonly known as "red pepper", "pepper", "hot red pepper", "tabasco", "paprika", and "cayenne" (*Costa et al.,* 2019).

It is the most important world spice crop (*Matthews and Jack, 2011*) and the second largest consumed spice throughout the world, after black pepper (Costa *et al.,* 2019).

It is widely cultivated in different regions of Ethiopia specifically, Amhara, Oromia, and Southern Nation Nationalities and Peoples Regional State. The total production of red pepper in Ethiopian for the year 2011/12 main cropping season (*Meher*) was estimated at 1,790,283 quintals (*Muchie, 2021*).

In Ethiopia, the total area under hot pepper production for dry pod has enhanced to 174,463.62 hectares with the total production of about 3,131,154.28 quintals (*CSA, 2020).* The total production of red pepper in the country for the year 2019/20 increased in 1.35% than the production year of 2018/19. Vegetables took up about 1.64% of the area under all crops at national level. However, of the total estimated area under vegetables,

the lion share which is about 73.13% and 17.81% was under Red peppers and Ethiopian Cabbage, respectively (*CSA,2020*).

The world's production area of hot pepper is estimated at 1.8 million hectare with total production of 3.9 million tonnes (*FAO, 2016*). In spite of its importance, hot pepper production in Ethiopia is stayed as low with a national average yield of 5.9 t/ha for green pod whereas it was 1.8 t/ha for thedry pod (*CSA, 2020*).

Ethiopians have strong attachment to red pepper consumption, which has high value principally for its high pungency (*Zeru et al., 2023*). Red pepper is an indispensable flavouring and colouring ingredient in most Ethiopian traditional meals and for the common traditional sauce "Wot," whereas the green pod is consumed as a vegetable (*Taddesse et al., 2023*).

According to the Ethiopian Export Promotion Agency *(EEPA*), the average daily consumption of red pepper by an Ethiopian adult is estimated 15 g, which is higher than tomatoes and most other vegetables. In addition to having significant role in Ethiopians daily dish it also plays a vital role in the national economy (*Gobie, 2019*).Furthermore, in many households, pepper provides the only needed flavor to enhance the intake of otherwise bland diets.

In Ethiopia, pepper is the most dominantly grown spice and is a high value crop for household consumption and for sale both at domestic and export markets playing an important role in the national economy (*Strategy, 2010*). At household level, pepper is an indispensable ingredient in Ethiopian daily cuisine. It is widely used as paste or sauce and also to modify the color, flavor and aroma of almost every cuisine. Despite the role of pepper in household consumption, households' income and national economy, pepper production system has been facing food safety challenges from contamination of mycotoxins

Mostly pepper is the common ingredient of the daily dish of almost all Ethiopians. People consume pepper for intake enhancement as well as to supplement dietary household needs. Red pepper is a major spice or condiment and the vegetable crop produced by the majority of smallholder producers in the south, Oromia, and Amhara regions. Despite the fact that the significance of red pepper in Ethiopian economy and current income-

generating capacity of red pepper as compared to its magnificent potential in the country had not been given due attention (*EEPA,2003).*

Pepper is an important agricultural crop because of its economic importance, nutritional and medicinal value of its fruit (*saleh et al., 2018)*

Pepper makes a significant contribution to the Ethiopian households and national economy. However, production and marketing of pepper are constrained by different factors. The main constraints that contributed for low productivity of pepper in Ethiopia include lack of proper and adequate inputs, traditional production methods, and lack of research outputs on production techniques (*Mekdes et al., 2017*). As a result, the variation in market outlet choice will be expected. Likewise, marketing problem like storage facilities, transportation, linkages with traders, quality-controlling mechanisms, market information, and price settissngs are weak in the region and need to be further investigated (*BoA, 2004, cited in Abay, 2013*). Such constraints are aggravated by underdeveloped infrastructure and weak transport facilities.

Farmers in Ethiopia in general and in Amhara region in particular are affected by low producer's price, on the one hand, and high consumer's price, on the other hand (*Abay,2013*). Therefore, to solve production and marketing problems and to increase the contribution of pepper to generate additional income for producers and traders,

They are used extensively in food processing and formulation all over the world (Mckee, 1995). Spices, like to various other agricultural products, can be vulnerable to an extensive array of microbiological pollutants prior to, during, and following harvest (Mckee, 1995; Kocićtanackov et al., 2007). Several studies conducted recently have documented the natural contamination and presence of mycotoxins in spices (jacxsens and de meulenaer, 2016; garcia et al., 2018a; motloung et al., 2018; el darra et al., 2019; potortí et al., 2020).

The production system for pepper has been confronted with food safety issues due to mold deterioration and mycotoxin contamination in various nations, including Ethiopia, despite the importance of red pepper for national economy, income, and nutrition. The environmental (high humidity and high temperature) and processing circumstances of red

pepper make it extremely vulnerable to mold deterioration and the production of mycotoxin.

According to many reports, red pepper is one of the most vulnerable substrates to fungus attack, which can lead to the production of mycotoxins such as ochratoxin A (Ota) and afs (*Marín et al., 2009*).

Mycotoxins are secondary fungal metabolites found in foods that can harm consumers' health *(Karlovsky et al., 2016). Aspergillus, alteraria, claviceps, fusarium, penicillium, and stachybotrys are the most important mycotoxigenic fungi. Aflatoxins (afs), trichothecenes, fumonisins (fbs), and other mycotoxins* are the most common types. zearalenone (zea), ochratoxins, and ergot alkaloids (*Richard 2007*). These mycotoxins can contaminate foods at various points along the value chain (*Bennett and Klich, 200b3).*

It can be contaminated by two types per harvest flavius fungi and after harvest aspargilus fungi, before harvesting (pre-harvest contamination are The main factors include improper drying, inadequate storage, pest and rodent infestations, mechanical damage, contaminated Processing equipment, inappropriate packaging as a result, controlling the critical factors such as temperature, humidity (and/or water activity) during drying, storage, processing, packaging and transportation stages in order to prevent/minimize the risks of mycotoxin contamination (*Santos et al., 2008*).

As a result, determining the occurrence and management of Mycotoxin contamination in red pepper powder produced at households in Addis Ababa is required for the collection of consistent data at the national level. In our information, despite the importance of *red pepper in* Ethiopian domestic food systems, limited documented knowledge, attitude and handling practices and occurrence of mycothoxins exists for *red peppe in household.* Hence, this study was aimed to assess knowledge, attitude and practice of red pepper powder producing at households in AddisAbaba and the occurrence of mycothoxins in storage condition, in the processing area, handling of red pepper powder, healthy impacts of Mycotoxins and provide evidence for safety interventions and regulations.

1.2 Statement of the problem

Mycotoxins in foods are a serious problem in many parts of the world, undermining public health and development efforts. Consumption of mycotoxin-contaminated foods can result in stunted growth, liver and kidney disease, immune system suppression, and death in both humans and animals. (*Zain et al., 2011*)

Ethiopia has one of the highest per capita red pepper consumption rates in the world, with an estimated average adult intake of 15 grams per day (*EEPA, 2019; Gobie, 2019).* The majority of Ethiopian households process and store red pepper powder themselves, using traditional methods that often lack proper sanitary and preservation practices.

However, despite its importance, red pepper powder is highly susceptible to contamination by mycotoxins, particularly aflatoxins and ochratoxin A, due to favorable environmental conditions such as high humidity, warm temperatures, and poor post-harvest handling *(Costa et al., 2019; Zeru et al., 2023).*

Mycotoxins are all heat-stable and they are not destroyed by cooking and normal industrial processing and are threatening human and animal health and the 'country' economy (*Dey et al., 2022).* They are toxic secondary metabolites produced by fungi such as Aspergillus, Fusarium, and Penicillium, and are associated with a range of serious health effects including immunosuppression, liver damage, carcinogenicity, and neurotoxicityand economic losses (*Karlovsky et al., 2016; Richard, 2007; FAO, 2021).*

The recent rejection of hot pepper exports from Ethiopia by the European market, valued at over 10 million USD, due to unsafe levels of aflatoxins and ochratoxins, illustrates the significant impact of mycotoxin contamination on international trade (*EFDA, 2023; RASFF, 2023).*

Recent studies conducted in Ethiopia and elsewhere have identified red pepper powder as one of the most frequently contaminated spices, especially in household and informal market settings where drying, storage, and grinding practices are not adequately regulated (Garcia et al., 2018; El Darra et al., 2019; Potortí et al., 2020; Taddesse et al., 2023). Despite the proven risks, there is limited data on the actual levels of mycotoxin contamination in red pepper powder processed at the household level in Addis Ababa.

Moreover, there is a critical knowledge gap regarding household handling practices, awareness of mycotoxin risks, and hygienic processing conditions.

A recent study by Zeru et al. (2023) emphasized that household-level food processing in Ethiopia often overlooks food safety risks due to lack of awareness, resources, and training. Without evidence-based interventions, the population remains exposed to long-term health hazards.Therefore, assessing the occurrence of mycotoxins in household-processed red pepper powder and evaluating knowledge and practices surrounding its production is essential to inform public health policies, food safety regulations, and community-level interventions.

1.3 Objectives

1.3.1 General objective

Assess the level of mycotoxin contamination in red pepper powder processed at households in Addis Ababa

1.3.2 Specific objectives

- Evaluate the knowledge and practice of red pepper powder processors at households on issues related to mycotoxins.
- Determine the level of AFB1 ,AFB2,AFG1, AFG2, Ocratoxin A (OTA), Diacetoxyscirpenol , Beauvericin, Enniatin A, Enniatin A1, Enniatin B, Enniatin B1 on red pepper powder collected at the time of processing at households.
- Determine the level of AFB1 , AFB2, AFG1, AFG2 Ocratoxin A (OTA), Diacetoxyscirpenol , Beauvericin, Enniatin A, Enniatin A1, Enniatin B, Enniatin B1 on red pepper powder collected after six month of storage at households.

1.4. Significance of the study

The findings of this study can be used to develop evidence-based guidelines for households on safe handling and processing of red pepper powder. This can help reduce the risk of mycotoxin exposure and improve food safety in Addis Ababa. Additionally, the study can inform policy decisions on food safety regulations and interventions to address mycotoxin contamination in red pepper powder.

Overall, this study can have a significant impact on public health by raising awareness and providing evidence-based recommendations to reduce mycotoxin exposure in Addis Ababa.

2. LITERATURE REVIEW

2.1. Red pepper

The origin of Capsicum species is extended from Mexico in the North to Bolivia in the South of Latin America, where it has been part of human diet since about 7500BC (Purseglove et al., 1981). Spanish and Portuguese explorers spread pepper around the world. Pepper was introduced to Spain in 1493, England in 1548 and Central Europe in 1585. Then, from Europe it spread to Asia.

Currently the crop is produced in various countries around the world including India, China, Pakistan, Indonesia, Sri Lanka, Thailand and Japan in Asia and Nigeria, Uganda and Ethiopia in Africa. India and Indonesia have been the largest producers. Currently China is the main producer and exporter in the world.

Peppers are among the most popularly consumed spices around the world. In the American continent, Capsicum is described as one of the oldest cultivated plants, regarded as a prehistoric crop.

In the first Columbus expeditions, it was recorded that peppers were used by the Mayan civilization as a condiment and in medicinal preparations (*Lentz et al., 2024*).In Central and South America, the center of origin and diversity of Capsicum, the peppers were called “aji” or “chilli” by Amerindian civilizations (Cichewicz and Thorpe, 1996). By the early 16th century, Capsicum cultivation spread throughout Europe, Asia and Africa. Due to their pungency, they were associated with black pepper, Piper nigrum L., although the species are not related. After its diffusion, different terms were used to refer to *Capsicum* fruits and commonly known as “red pepper”, “pepper”, “hot red pepper”, *“Tabasco”, “paprika*”, and “*cayenne” (Suzukiand Iwai, 1984)*.red pepper is a horticultural crop produced worldwide. It belongs to one of the most valuable plant families known as Solanaceae, containing more than 3000 species that comprises many economically. Pepper has possibly the longest history of any vegetable crop in Ethiopia, according to

EEPA evidence (*EEPA, 2008*). There is no recorded evidence to back up the notion that the Portuguese brought it to Ethiopia in the 17th century (*Huffnagel, 1961*). C. frutescens and C. annuum peppers are highly popular in Ethiopia. The spicy chilli pepper (Capsicum frutescens) is grown across Ethiopia and is a staple in traditional Ethiopian cuisine throughout most of the country's flora. The C. annuum plant produces fruits known as "Beyayenetu," which are consumed both raw and cooked. Mature and spicy fruits are ground into a powder and dried before being combined with a spice blend known as "Berbere." Pepper and hot sauce are commonly used in Ethiopian cookery.

In Ethiopian cooking, pepper and the hot sauce called "Awazie" are both frequently used ingredients. In fact, pepper may be the vegetable with the longest history in the nation (Hedberg et al., 2006)



Figure 1 dried and ground red pepper

2.2. Red pepper production

Pepper is produced in all the continents except Antarctica. It is believed to have originated in Central and South America. It have been Introduction into Asia and Africa in 1493 (Bosland and Votava, 2000) . Over 48% of the world pepper is produced in Asia, China being the leading country (*Rehima, 2006)*. Peppers is the world's second important vegetable ranking after tomatoes and it is the most produced type of spice flavoring and color to food while providing essential vitamins and minerals. The nutritional value of hot pepper merits special attention. It is a rich source of vitamin A and E. Both hot and sweet peppers contain more vitamin C to prevent flu colds than any other vegetable crop *(Boselad and Votava, 2000).*

Its unusual flavor, scent, color, and pungency, as well as the variety of ways to consume it, have made this spice popular in gastronomy. According to the Food and Agriculture Organization (FAO) in 2016, the global production area for dried Capsicum was 1,798,847 hectares, with an annual yield of 3,918,159 tons. China is a major producer of crushed and ground peppers. China produced the most peppers in 2016, followed by Mexico and Turkey. The production indices demonstrate the importance of the Capsicum crop, particularly in developing economies.

However, the fact that they are regularly contaminated with rotting fungi has put the income of small businesses at danger as well as large-scale pepper farmers. Peppers are among the spices most prone to fungal infection, particularly by possibly mycotoxigenic species. The majority of these goods reach the market without being properly processed. In general, they are just dried and powdered before being packed and sold to the public (Kneifel et al., 1994).

The whole pepper production chain should be managed carefully to prevent fungal infection and mycotoxin contamination.. In the sowing stage, excessive irrigation and fertilizer application above the recommended levels enhance plant susceptibility to fungal colonization (Richardson et al., 2018). The selection criteria for pepper seeds must be stringent; however, this is not always the case because most pepper seeds are produced by small rural farmers. Capsicum fruits are harvested at various stages of ripeness. They are often harvested completely grown for the agro-industry sector (Toontom et al., 2012).

The pod is vulnerable to mechanical injury and the action of phytophagous insects, which are occasionally transmitters of fungal spores, at this stage of advanced maturity. Furthermore, it is recommended that red pepper moisture content (65-80% = 0.995 aw) be quickly decreased to around 13% (=0.50 aw) by drying after harvesting to suppress enzyme activities, fungal development, and mycotoxin contamination (Sanzani,. 2016).

The drying and storage phases are critical in the pepper production chain. Traditionally, peppers are dried by direct sun exposure or mechanical heat drying; these methods involve extended periods of time at oscillating temperatures and humidity or at higher controlled temperatures for short time periods, respectively (*Kaleemullah et al,. 2005*). Due to its low operational cost, pepper drying under the sun remains the most widespread method used in Asia, Africa and Central/South America. After being placed on the floor and several times to obtain an even drying, the pepper pods are usually smoked (*FIA 2010).*.

In China and Nigeria, artisanal drying of pepper can take between 7 and 28 days. In Chile, In the pepper production chain, the drying and storage phases are crucial. Traditionally, peppers are dried by direct sun exposure or mechanical heat drying; these processes need extended periods of time at varying temperatures and humidity or shorter times at higher controlled temperatures (*Kaleemullah et al, 2005*). Pepper drying under the sun is still the most common method used in Asia, Africa, and Central/South America due to its low operating costs. The pepper pods are often smoked after being set on the floor multiple times to ensure even drying (*FIA 2010*). Artisanal drying of pepper can take 7 to 28 days in China and Nigeria. In Chile, for example,

The procedure is carried out using traditional procedures. After sun drying, peppers are placed in a traditional "pidil" structure constructed of light wood (similar to bamboo). Pepper is then smoked within traditional homes constructed of wood and straw known as "ruca" *(FIA 2010).* Capsicum annuum var. aviculare (locally known as "*chiltepn"),* a wild pepper, is dried in the sun for 5 to 7 days in Mexico (*Montoya-Ballesteros et al,. 2014*).Furthermore, depending on the weather, sun drying takes between 14 to 21 days in other countries. Pods are impacted by temperature variations, dust exposure, wind, and

insect infestation during the drying process. This interval permits fungal colonization, particularly by xerophilic/xerotolerant fungi (*Aspergillus and Penicillium* species). Furthermore, inadequate post-harvest cleanliness in storehouses might result in additional fungal colonisation and an increase in the risk of mycotoxin contamination (*Santos et al, 2008).* Water can be removed mechanically by hot air exposure (e.g., wind chambers), sublimation (freeze-drying method), or water molecule friction generated by a magnetic field (e.g., microwave drying). Mechanical dehydration is utilized on an industrial scale despite high energy costs; the temperature, air velocity, and humidity regulated conditions minimize the time for microbial contamination and increase pepper quality *(Hossain et al., 2003).* Packaging is the final stage of pepper production (Figure 1). For pepper samples, polyethylene bags and vacuum-packaging methods have proven to be more efficient for inhibiting water reintroduction and decreasing aeration, both factors that are important for limiting fungal growth and mycotoxin production.

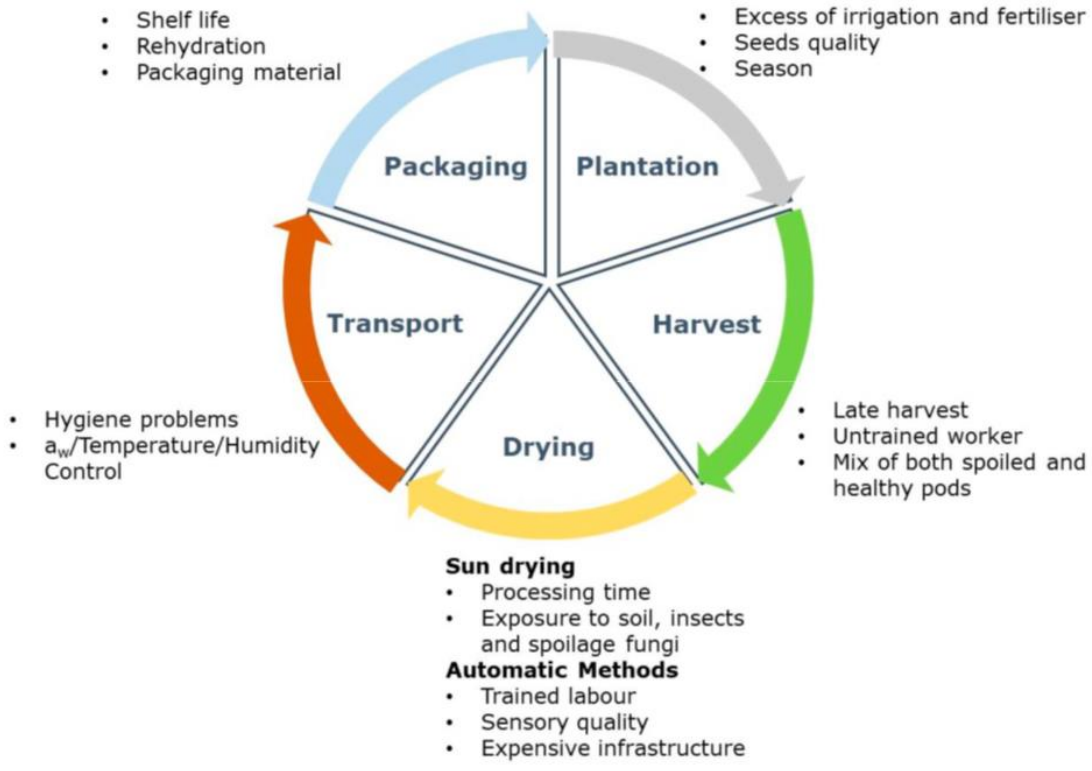


Figure 2 critical factors favoring fungal growth and mycotoxins contaminations during the pepper production chain

2.2.1 Production and consumption of red pepper in the world

Pepper is grown in tropical and subtropical Africa, Asia, Southern and Central Europe, and Northern America, among other places (Thampi, 2004).

Global production of "Chillies and peppers, red (*Capsicum spp. and Pimenta spp*.)" reached a record 36.97 million metric tons in 2022. This indicates a continued upward trend from the 36 million tons reported in 2017 for fresh types. While a specific updated figure for dry types globally is not as readily available in a single aggregated number from FAOSTAT, the overall trend suggests an increase in dried pepper production as well, driven by strong demand for spices.

The surface cultivated area has also continued to expand. While the precise global figure for 2022/2023 for all pepper types is not consistently reported in easily accessible summaries, the 2022 production volume of 36.97 million metric tons was produced on 2,020,816 hectares. This is a significant adjustment from the "roughly 3.8 million hectares" previously estimated across all pepper types, suggesting possible refinements in reporting or a greater focus on specific classifications in recent data.

In *2022*, China produced 16.81 million metric tons of peppers, accounting for approximately 45.47% of the world's total production. Other significant producers in 2022 include Indonesia (3.02 million metric tons), Turkey (3.02 million metric tons), and Spain (1.53 million metric tons). The top argest producers (China, Mexico, Indonesia, Turkey, and Spain) together accounted for about 74.36% of the world's pepper production in 2022. .

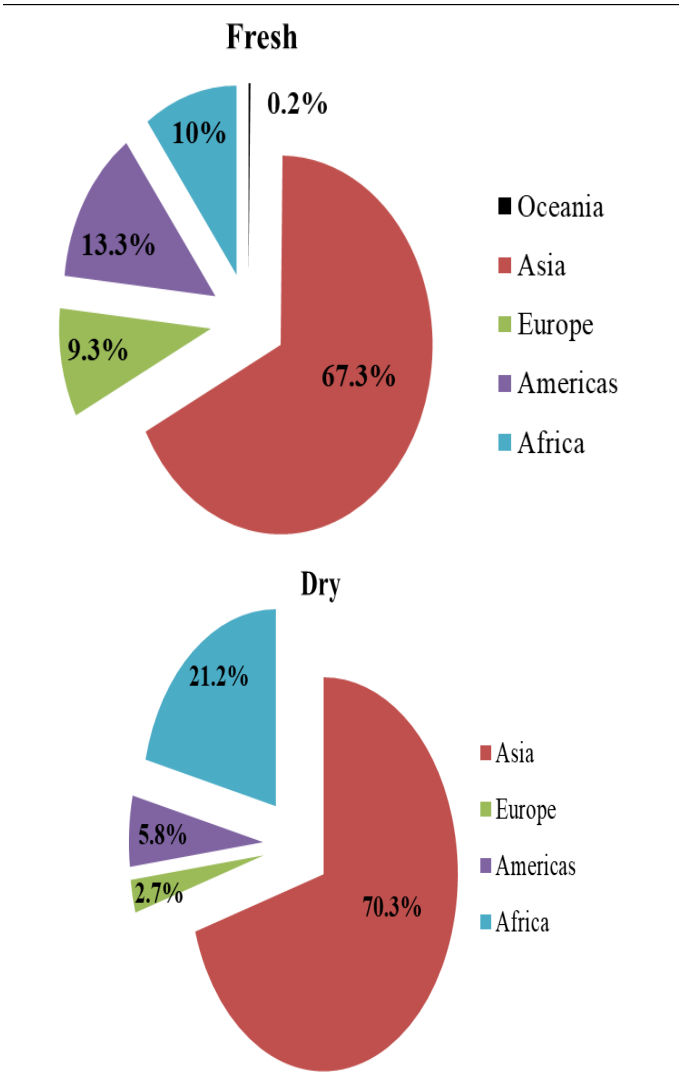


Figure 3 Regional share global production of fresh and dry pepper.

Global pepper consumption is estimated about 398,000 tons and it is growing rapidly. China and India are the top consumers, with about 49,000 and 59,000 metric tons, respectively, in 2014 (Nedspice, 2015). Global consumption rate is 2-3% and Asia and Middle East have a consumption rate of 3–4%. Moreover, red pepper is widely consumed nearly by 25% of the global population on a regular basis (Bode and Dong, 2011; Sharma et al., 2013). Valued for its color, aroma, taste and pungency, red pepper is commonly used to alter the color and flavor of stews, soups, sausages, snacks, cheese, sauces, pizzas, salad dressing and confectionary products, as well as for pharmaceutical or physiological functions (Nieto-Sandoval et al., 1999; Li et al., 2009; Topuz et al., 2009). Moreover, there is an increasing trend with the economic value of pepper production

since 1991, making it a good source of income for producers in several countries and playing a key role in the global trade. Recently, it is reported that dry pepper worth 3.8 billion US dollars, whereas fresh pepper worth 30,208 billion US dollars (FAOSTAT, 2017). An increasing economic value of four and six times higher in dry pepper and fresh pepper was recorded over the past 25 years, respectively.

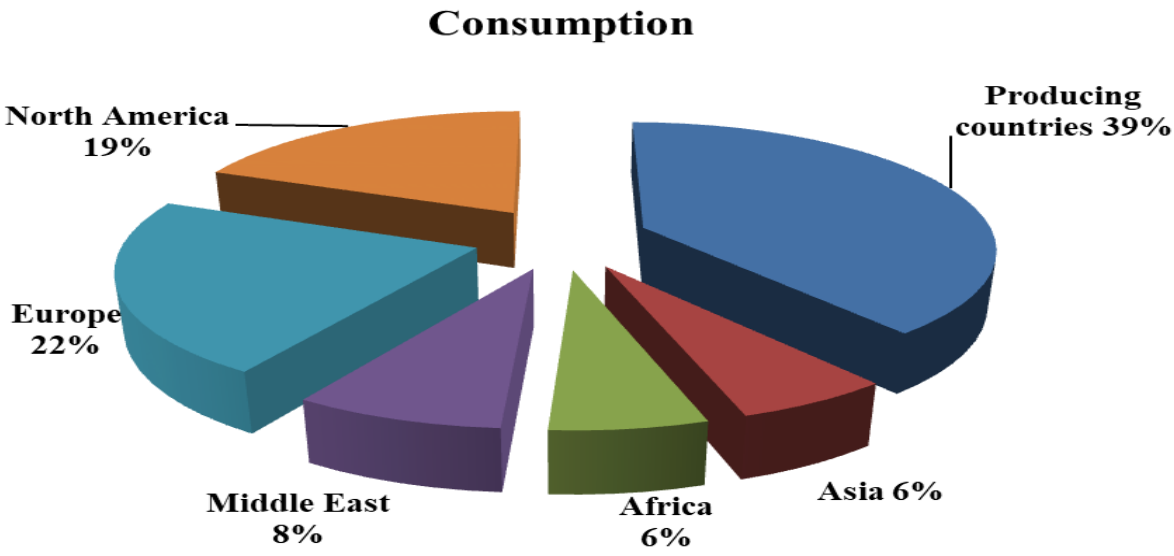


Figure 4 Global consumption of pepper by region *Source: Nedspice (2015)*

Table 1:Top 10 Countries in Pepper Production –in the world Source FAO(**2022/2023)**

Rank	Country	Production Volume (Metric Tons)	Year	Notes
1	China	20,559,000	2023	Leading producer, often includes both fresh and dried forms.
2	Mexico	3,685,900	2023	Major producer and exporter, especially for fresh peppers.
3	Indonesia	2,956,000	2023	Significant producer in Southeast Asia.
4	Turkey	2,625,669	2022	High production, much of it consumed domestically.
5	Spain	1,494,000	2023	Leading European producer, particularly for bell peppers.
6	Egypt	813,280	2023	Growing production, including for export.
7	Nigeria	761,240	2023	Important producer in West Africa.
8	Algeria	767,420	2023	Significant North African producer.
9	United States	577,080	2023	Notable production, with key states like Florida and California.
10	Tunisia	484,600	2023	Another important North African producer.

2.2.2 Red pepper production in Ethiopia

The total production of red pepper in Ethiopian for the year 2011/12 main cropping season (*Meher*) was estimated at 1,790,283 quintals (Muchie, 2021). The total production of pepper in the country for the year 2013/2014 Ethiopian main cropping season (Meher) was estimated at 2.8 million quintals. In this year the total cultivated land and production was 61,069 hectare and 1, 580,066 quintals respectively CSA (2013/2014). The total amount of crop produced in 2014/2015 production year was 92,455.73 hectare and 1,707,656.64 quintals CSA (2014/2015). Red pepper production system for green pod and dry pod has stayed as low input and low output with a national average yield of 7.6 ton/ha and 1.6 ton/ha, respectively (Gobie, 2019).

Growing pepper requires well drained and rich in organic matter soil, as well as 600-650 mm rainfall (Berhe et al., 2022). It grows well on well-prepared soil that is free from perennial weed. Depending on the area, harvesting starts 5 to 6 months from transplanting. Planting is carried out in the beginning of the main rain season. The red pepper is harvested when it is fully red and starts to dry. After harvesting, it is dried and shaded for high-quality oleoresin (Gobie, 2019).

In terms of consumption, Ethiopians have a marked preference for dark red pepper and is valued essentially for its high pungency. The ground product is an important coloring and flavoring component in many traditional food items including ―wot, while the green pod is served as a vegetable alongside of other foods. Apart from the key role in daily diet, cultivars with deep red color and large pods have higher demand in the commercial market for industrial processing and to some extent, Ethiopia exports red pepper extracts to Europe (*Vijayalaxmi and Sreepada, 2014).*

The total production of red pepper in the country for the year 2005/06 Ethiopian main cropping season (Meher) was estimated at 1,790,283 quintals. Rain-fed pepper production in 2005/06 production season was 777,602 quintals in South Nations Nationalities and People Regional State (SNNPRS). This accounted for 43% of the country's production *(CSA, 2006).* In Ethiopia, the production of red pepper is constrained by variable seasonal conditions.

The 2005/06 yield of pepper was very high, around 22qt/ha in the country, which was greater than the previous years" yield (13quntal per hectare). The Ethiopian Export Promotion Agency (EEPA (Ethiopian Export Promotion Agency), 2003) also carried out a spice potential market study in Amhara, Oromia, and SNNPRS, and identified that the land coverage for pepper in the three regions. According to the study results, pepper production accounts for 34% of the total spices production in the three regions. Despite the fact that the red pepper production system for green and dry pod has stayed as low input and low output with a national average yield of 7.6 ton/ha for green pod whereas it was 1.6 ton/ha for the dry pod, respectively (*Central Statistical Agency, 2006).* The decline of red pepper production is also attributed to poor varieties, poor cultural

practices, the prevalence of fungal or blights and bacterial as well as viral diseases (Fekadu & Dandena, 2006).

2.3 Mycotoxins in red peppers

Mycotoxins are low-molecular-weight secondary metabolites produced by filamentous fungi. The word mycotoxin is derived from the Greek radicals “mykes” and “toxicum”, meaning fungus and toxin, respectively. This term was coined after a veterinary outbreak in England in 1962, when approximately 100,000 turkey poultry died from the ingestion of aflatoxin-contaminated peanut meal (*Stevens et al., 1960*).

Since then, mycotoxins have become an important issue in relation to the food safety requirements for international marketing of agri-food commodities for human and animal consumption. Mycotoxigenic fungi grow in a wide range of agriculture crops (e.g., cereals, soybeans, grapes, tree nuts, groundnuts, coffee, cocoa and spices) and can produce one or more mycotoxins (e.g., water activity (aw), temperature, pH, and nutritional substrate) are the key determinants of fungal colonization and mycotoxin biosynthesis (Schmidt et al., 2008).

Capsicum peppers are condiments widely used in cookery. As with any other agricultural crops, they are also susceptible to fungal infection and mycotoxin contamination. In the field, the phyllosphere of the growing plants are mainly colonised by yeasts and by the filamentous fungal genera Alternaria, Fusarium, Cladosporium and Rhizopus. In the harvesting and post-harvest phases, including drying, and subsequent product transportation, Aspergillus and Penicillium species are the predominant contaminants (Mandeel.2005). Mycotoxins are heat stable and difficult to remove once present in the Capsicum pepper production chain. Previous studies have shown that industrially processed Capsicum products can be contaminated with *aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FBs), zearalenone (ZEN), trichothecenes (TCTs), and patulin (PAT) (Santos et al.,2011).nivalenol, T-2 toxin, HT-2 toxin,Zearalanone and fumonisinB1,α–Zearalenol,βZearalenol,Diacetoxyscirpenol,3AcetylDeoxyninvalenol,15Acetyl,Deoxynivalenol,Deoxynivalenol,FB1,FB2,FB3, Fusaric Acid, Moniliformin, Ochratoxin A, Beauvericin, Enniatin A, Enniatin A1, Enniatin B, Enniatin B1* chilli pepper, black pepper, white pepper.These mycotoxins can trigger a number of acute and chronic

diseases and, in more serious cases, can result in fatal consequences (e.g., most recently in Tanzania in 2016) (*Kamala et al., 2018*).

The European Commission (*EC*) established the most rigorous legislation for mycotoxin in food and in feed, including regulations for AFs in *Capsicum* fruits with maximum tolerable limits (MTL) set at 10 μg/kg for total *AFs (AFB1 + AFB2 + AFG1 + AFG2)* and at 5.0 μg/kg for AFB1 (European Commission Regulation 2012).

The regulation was recently updated with the maximum levels of OTA in spices of 20 μg/kg for Capsicum powder and 15 μg/kg for mixtures of Capsicum with other species (European Commission *Regulation 2015*). No maximum tolerable Fusarium toxin or PAT concentration has been established for pepper powder to date. Apart from EC regulations, other countries have their own legislation, generally less comprehensive and restrictive.

In Chile, the regulation for mycotoxin in spice, including Capsicum, establishes MTL set at 10 μg/kg, only for total AFs. Similarly, in Uruguay the legislation is established only for aflatoxins, with MTL set at 10 μg/kg for total AFs and at 5.0 μg/kg for AFB1. In Brazil, current legislation includes MTL established for total AFs (20 μg/kg) and OTA (30 μg/kg) in Capsicum spp. (dry fruit, whole or crushed, and mixtures of spices). In Pakistan, there is regulation for AFs in chilli powder with MTL fixed in 15 μg/kg for total AFs, 10 μg/kg for AFB1 and, 7 μg/kg for OTA. As for Capsicum fruits, the MTL is set at 30 μg/kg for total AFs (FAO 2018). In addition, Mexico has no legislation to control AFs and OTA in chili, a cause for concern, considering that this country is among the major exporters and consumers of Capsicum. Capsicum peppers are commodities of economic relevance that are used in different gastronomic cultures. However, there is still a lack of information, especially on the fungus-mycotoxin-Capsicum pepper relationship. This review aims to summarise and discuss the current status of filamentous fungi and mycotoxins in Capsicum products, including the role of ecophysiological factors in mycotoxin contamination of these products.

2.3. Main Sources and Mycotoxins

Pre-harvest contamination primarily occurs when crops are in the field, growing and developing. The main factors include: Fungal Infection in the Field: Airborne Spores: Fungi release spores that are dispersed through the air and can land on the crop.

Soilborne Fungi: Some fungi reside in the soil and can infect plant roots or other plant parts. Insect Damage: Insect feeding creates wounds and entry points for fungi to invade the plant.Rain Splash: Rain can splash fungal spores from the soil onto the plant, causing infection. Contaminated Seeds: Fungal spores on or in seeds can lead to plant infection and subsequent mycotoxin production. Environmental Factors, Warm Temperatures: Many mycotoxin-producing fungi thrive in warm climates. High Humidity: High moisture levels, whether from rainfall or humidity, are essential for fungal growth and mycotoxin production Drought Stress: Drought and other stress conditions can weaken plants, making them more susceptible to fungal infection.Weather Variations: Fluctuations in temperature and moisture, including prolonged wet or dry spells, favor fungal growth and mycotoxin accumulation.

Agricultural Practices:Poor Crop Rotation: Planting the same crop repeatedly can increase fungal populations in the soil. Inadequate Pest Control: Lack of pest management can increase insect damage, providing entry points for fungi Poor Soil Health: Nutrient imbalances can stress plants and make them more prone to infection. Excessive Nitrogen Fertilization: May increase plant susceptibility to fungal diseases in some cases. Delayed Harvest Delaying harvest exposes the crop for longer periods to favorable conditions for fungal growth.

After Harvesting (Post-Harvest Contamination): Post-harvest contamination occurs when crops are harvested, transported, stored, and processed. include: Improper Drying, Slow Drying: Inadequate airflow and slow drying rates allow fungi to multiply on the crop.Uneven Drying: Uneven drying can create areas with high moisture content where fungi can thrive.Inadequate Storage:High Humidity:Storage areas with poor ventilation, high moisture, and inadequate climate control promote fungal growth.

Temperature Fluctuations: Fluctuating temperatures can create moisture condensation within the storage facility, creating favorable conditions for

fungi Contaminated Storage Facilities: Poorly cleaned storage facilities can harbor fungi that will contaminate newly stored crops. Pest and Rodent Infestations Insect Damage: Insects can damage stored products, creating entry points for fungi Rodent Activity: Rodents can damage bags, packaging, and the product itself, also creating entry points for contamination.Mechanical DamageCracked and Damaged Grains: Cracked grains are more susceptible to fungal growth due to increased surface area exposure. Improper Handling: Rough handling during harvest, transport, or processing can cause damage and create entry points for fungi.Contaminated Processing Equipment:Poor Cleaning: Improperly cleaned milling equipment or other processing equipment can harbor fungal spores. Inappropriate Packaging:Lack of Barrier Protection: Packaging that does not protect against moisture can lead to contamination. Reusable packaging:that is not sanitized is a good.

However, in special cases like under unusually hot or dry conditions *Aspergillus* and *Peni cillium* species can also affect crops during the growing season. On the other hand, field fungi can continue growing and produce mycotoxins during transport and storage. Mycotoxins cause economic losses at all levels of food and feed production, including crop and animal production, processing and distribution. According to the FAO (Food and Agriculture Organization) around 50% of the world's crop harvests may be contaminated with mycotoxins .there are different types of mycotoxins some of them are as follows.

2.4. Types of mycotoxins

Mycotoxin producing fungi can be divided into two groups. Field fungi (such as *Fusarium sp.*) typically produce mycotoxins in the field ("pre-harvest") and Storage fungi (such as *Aspergillus* and *Penicillium sp.*) typically occur after harvest ("post-harvest") It can be occur both before and after harvest. Understanding the different sources and mechanisms of contamination at each stage is crucial for effective prevention.

2.4.1 Aflatoxins (B1, B2, G1 and G2)

The word "mycotoxin" is derived from two words; "mukes" referring to "fungi" (Greek) and "toxicum" referring to "poison" (Latin).The word "Aflatoxin" is the combination of three words; "a" for the Aspergillus genus and "fla" for the species flavus, toxin for poison. Aflatoxin species have polycyclic structure; G series Aflatoxins are six-member lactones and B series Aflatoxins are pentanone derivatives (*Bakirdere S. et al, 2012).*

The four major naturally occurring aflatoxins are aflatoxins B1, B2, G1 and G2. Aflatoxin B1,B2, G1 and G2 belong to a group of around 20 similar chemicals. The letters 'B' and 'G' stand for the blue and green fluorescent colors produced by these compounds when exposed to UV light, respectively, while '1' and '2' stand for the major and minor component.(*Ketney O. et al, 2014).*

A Only 'B' aflatoxins are produced by A. flavus (AFB1 and AFB2). Both 'B' (AFB1 and AFB2) and 'G' (AFG1 and AFG2) aflatoxins are produced by A. parasiticus. A. nomius' status looks to be comparable to that of A. parasiticus. Aflatoxin B1 is the most frequent aflatoxin in foods, and it's also the one that's been examined the most in toxicological studies. A. flavus is widespread in the environment, but A. parasiticus is much less so (Cressey et al, 2009). Tropical climates with high temperature and humidity are suitable conditions for mycotoxin contamination and spices are mostly produced in countries with tropical climates. Spices are exposed to a wide range of microbial contamination as a result of improper production process, extended drying times and poor storage conditions and red pepper flakes is a very sensitive product for aflatoxin formation depending on unsuitable processing conditions because they are usually dried on the ground in the open air (*Gulderen et al, 2012*). The hot and humid climate, production conditions with extended drying times, and often-inadequate instructions to the farmers, may cause

considerable quality problems (*Aydin et al, 2007).*The drying and storage phases are critical in the pepper production chain. Traditionally, peppers are dried by direct sun exposure or mechanical heat drying; these methods take extended periods of time at different temperatures and humidity or at higher pcontrolled temperatures for short time periods, respectively. The whole pepper production chain should be managed carefully to prevent fungal infection and mycotoxin contamination (*Costa., et al, 2019*). The most important fungi able to produce toxin are *Aspergillus, Fusarium and Penicillium* species. From various kinds of toxins the most important which are produced by these fungi are aflatoxins, ochratoxins, zearalinon and others.

Those toxins are highly toxic compounds that cause many kinds of diseases including cancers. Although fungi growth requires moisture, they have the capability to grow on all foods without an exception, whether their moisture content was high or low. Fungi grow on crops in the field, during harvest, after crop harvest and storage (*Essawet N.et al, 2017).*Fungi also grow within wide range of temperatures (15- 35 ºC) and cause the damage of physical (in shape, texture, color, aroma and taste) and chemical (due to the fungal consumption of nutrients so lowering the food content of organic matter) changes of foods in addition to the aflatoxins that they secret.

It is not necessarily means that every fungi infected commodity is containing aflatoxins because fungal growth needs conditions different from that needed to produce toxins such as the moisture of the infected commodity, medium temperature, medium content of oxygen and other required conditions for fungi to grow and produce toxins. However, not every fungus has the genetic capacity to produce aflatoxins even he belongs to one species known with aflatoxins production due to the differences between the isolated strains from the same species which are accompanied with differences in the capability of toxin(s) production according to the genetic capacity *(Essawet N. et al, 2017).* And among all mycotoxins, AFB1 is considered to be the most carcinogenic (*Chauhan., et al,2016*). Aflatoxins, each of which is a group of closely related mycotoxins, may be produced by three of *Aspergillus* species (i.e.A. *flavus*, A. *parasiticus*, and the rare A. nomius) which contaminate plants and plant products. Aspergillus flavus produces merely the B type of Aflatoxin, while the other two species produce both A flatoxins B (B1,B2), G (G1,G2).

The levels of aflatoxins, including AFB1, in red pepper samples from the Amhara region of Ethiopia. It reported the presence of AFB1 and AFG1 in red pepper samples.The study found that a significant proportion of the contaminated red peppers had total aflatoxin levels exceeding the maximum permissible levels set by the European Union. It conducted a risk assessment, highlighting the potential health risks associated with the consumption of aflatoxin-contaminated red pepper. (Hunduma et al., 2020).

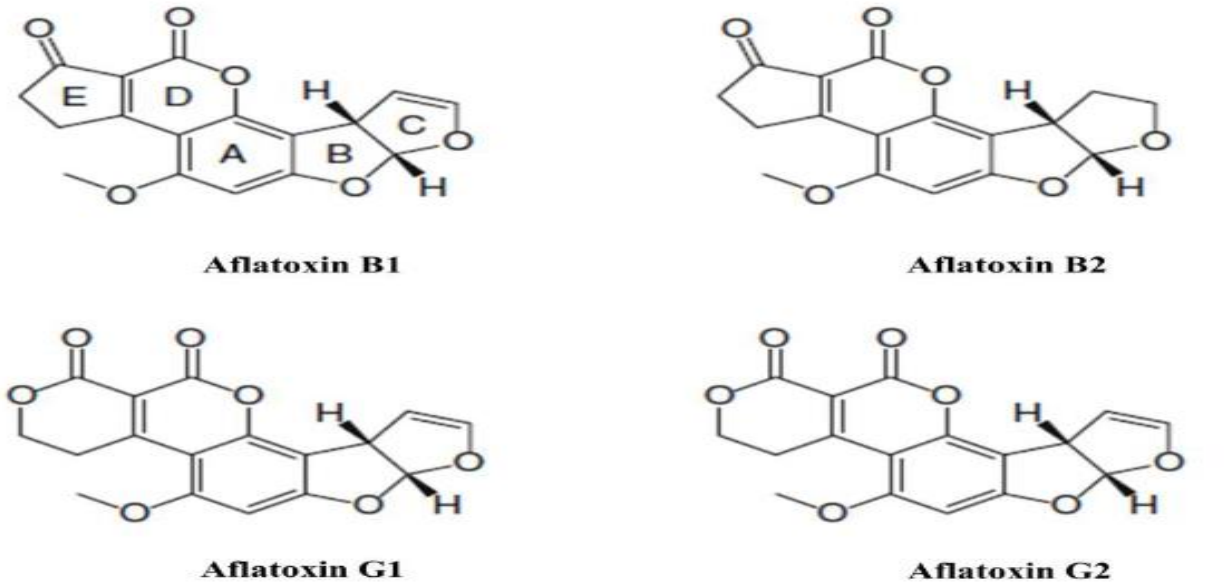


Figure 5: Chemical structures of aflatoxin B1, B2, G1 and G2. Source: da Rocha et al. (2014)

2.4.2 Ochratoxin A

Ochratoxin A is produced by numerous species of *Aspergillus*, involving *Aspergillus alliaceus,Aspergillus auricomus, Aspergillus carbonarius, Aspergillus glaucus, Aspergillus melleus*, and *Aspergillus niger* as well as *Penicillium verrucosum (Yu and Pedroso, 2023)*. It is a common food-contaminant defined as a potent nephrotoxin to all animal species (*Gurikar etal., 2023).* Following AFs, OTA is the most prominent mycotoxin found in pepper samples. Due to its immunotoxic action, nephrotoxic and carcinogenic potential, OTA contamination is of global concern. Overall, OTA is the most common mycotoxin found in food and feed. OTA has previously been reported in red pepper samples (*Ham et al.,.2016*). Geographical, climatic characteristics and crop management systems directly affect mycotoxin contamination levels. (*Almela et al).*and its EU established level is 15 µg/kg.

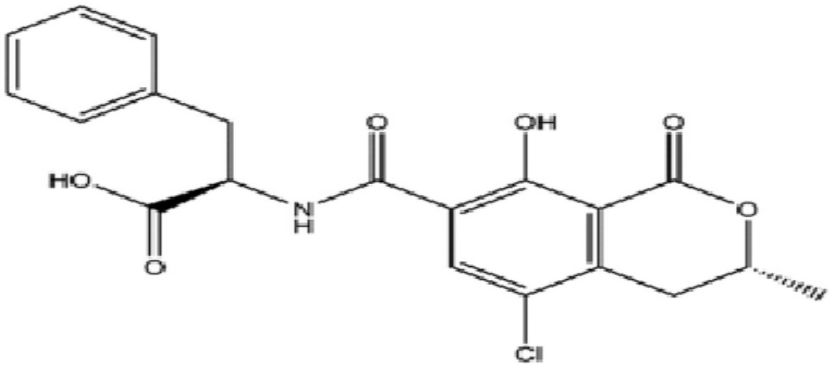


Figure 6 Chemical structure of ochratoxin A.

2.4.3 Diacetoxyscirpenol (DAS)

Diacetoxyscirpenol (DAS) is a type A trichothecene mycotoxin, primarily produced by *Fusarium* species such as *F. langsethiae*, *F. poae*, and *F. sporotrichioides* (Liu et al., 2025; Pan et al., 2024).It is known for its high toxicity and can contaminate various food and feed commodities, particularly cereal grains (wheat, sorghum, maize, barley, and oats), as well as potato products, soybeans, and coffee (Pan et al., 2024). DAS poses a significant risk to human and animal health, often causing hematological disorders and gastrointestinal issues, including emesis (Liu et al., 2025). Due to its toxicity and co-occurrence with other *Fusarium* mycotoxins, continuous monitoring and robust risk assessments are crucial (Kolpakova et al., 2024; Pan et al., 2024)

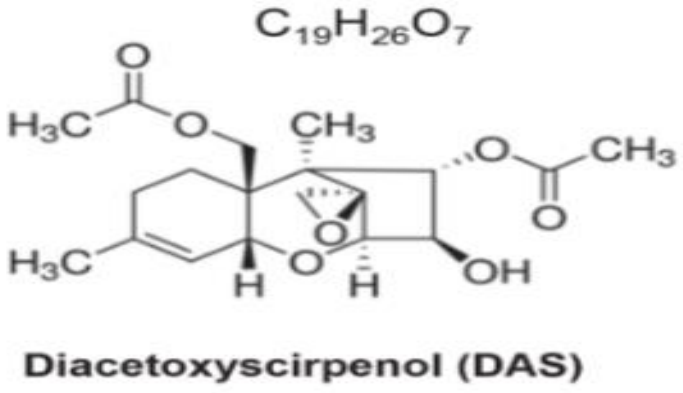
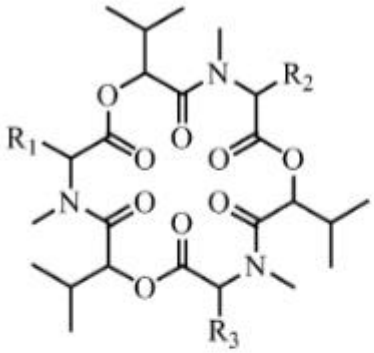


Figure 7 chemical structure of Diacetoxyscirpenol

2.4.4 Enniatins

Enniatins are secondary fungal metabolites that have been known to science for several decades, and recent reviews continue to highlight their significance (*Kowalska et al., 2017; Sun et al., 2025*). They represent six-membered cyclic depsipeptides, which are commonly composed of three alternating D-hydroxyisovaleryl and three N-methyl-L-amino acid residues. Individual enniatins are distinguished by the nature of the N-methylamino acid residue, and more than 10 distinct enniatins have been either isolated or synthesized so far (*Pan et al., 2024; Sun et al., 2025*). Enniatins are cationophoric compounds that possess insecticidal activity (Kowalska et al., 2017; Sun et al., 2025) and were found to be potent and specific inhibitors of acyl-CoA: cholesterol acyltransferase (ACAT) activity (*Sun et al., 2025*). Beauvericin, which is the only enniatin that is composed of N-methyl-L-phenylalanyl residues, was subject to more profound toxicological investigations in recent years (Sun et al., 2025). It was discovered that the metabolite was produced by *Fusarium subglutinans* and various other *Fusarium* species (Logrieco et al., 1998; Sun et al., 2025). Importantly, contamination limits for enniatins and beauvericin are not yet fully established by the European Commission, although data collection for risk assessment is ongoing (Mérieux NutriSciences, 2024; Zizzadoro & Nardella, 2024).



Compound	
Beauvericin	R_1=R_2=R_3= -$CH_2C_6H_5$
EnniatinA	R_1=R_2=R_3= -$CH(CH_3)CH_2CH_3$
Enniatin A1	R_1=R_2= -$CH(CH_3)CH_2CH_3$, R_3= -$CH(CH_3)_2$
Enniatin B	R_1=R_2=R_3= -$CH(CH_3)_2$
Enniatin B1	R_1=R_2= -$CH(CH_3)_2$, R_3= -$CH(CH_3)CH_2CH_3$

Figure 8 The chemical structures of beauvericin,enniatin (A,A1,B and B1):

Table 2: Major Mycotoxins and their toxicities

No	Mycotoxin	fungal Species	Toxicities
1	Aflatoxins(aflatoxins B1, B2, G1, G2,	*Aspergillus parasiticus,Aspergilus flavus,Aspergillusbombycis, A.pseudotamarii, A. nomius, etc*	Liver cancer; hepatocellular carcinoma; target DNA; mutagenic and teratogenic effects
2	Ochratoxins (ochratoxins A, B,C)	*Species of Aspergillus and Penicillium, including Aspergillus ochraceus,Aspergillus niger, Aspergillus carbonarius, Penicillium verrucosum*	Immunotoxicteratogenic, neurotoxic,hepatotoxic, nephrotoxic in humans, ochratoxin A was linked to urothelial tumors, chronic interstitial nephropathy, renal failure, and Balkan endemic nephropathy; etc.
3	Trichothecenes (trichothecene mycotoxins), examples include deoxynivalenol (vomitoxin), 3- and 15 acetyl deoxynivalenol,nivalenol, anguidine, T-2 toxin, HT-2 toxin, crotocin, diace-	*Species of Fusarium (Fusarium crookwellense, F culmorum, F graminearum,Fpoae),Myrotheciu mVerticimonosporium,Trichotheci um,Trichoderma,Cephalosporium, Stachybotrys, and Spicellum*	They can diffuse into cells and block translation by interacting with eukaryotic ribosomes; this is their primary action mechanism. Other action mechanisms for toxicity include inhibiting DNA, RNA, and protein synthesis, lipidperoxidation, apoptosis, inhibiting mitochondrial functions, neurotransmitters changes, and cytokine activation. Exposure to trichothecenes affects nearly all key systems in vertebrates . . . alimentary toxic aleukia (ATA) in humans, etc.

	toxyscirpenol, macrocyclics, etc.		
4	Zearalenone (formerly referred toas *F-2* toxin	*Species of Fusarium,* such as *Fusarium crookwellense, Fusarium cereals, Fusarium semitectum, Fusarium equiseti, Fusarium graminearum, Fusarium culmorum*	Zearalenone or its metabolic compounds are known to bind transcription factors, including pregnane X receptors involved in expressing enzymes in pathways of biosynthesis; zearalenone chronic administration can cause uterine fibroids, pituitary adenomas, hepatocellular carcinoma, and liver

2.5 Impact of mycotoxins' contamination on health, trade and economy in Ethiopia

Mycotoxins may have acute or chronic health consequences for people. AFs, one of the most common mycotoxins, have well-documented health effects in Africa. For example, several hundred Kenyans died from acute AFs poisoning between 2004 and 2006 (Jalili and Jinap, 2012; Iqbal et al., 2013). Stunting, immunosuppressive effects, and cancer are some of the chronic health consequences associated with AF exposure.(*Ozbey and Kabak 2012; Tosun and Ozden 2015).*Since 2002, AFs have been classified as Group 1 human carcinogens by the IARC. Synergistic effects with HBV can lead to chronic liver disease (CLD) or hepatocellular carcinoma (HCC) (*Kneifel and Berge, 2022; Çağindi and Gürhayta, 2016; Hierro et al. 2008*). A quantitative AFs-related HCC risk assessment performed by (Liu et al. 1994). based on nation's food consumption patterns, AFs food prevalence, HBV prevalence, and population size revealed that AFs exposure can be

attributed to 25,200–155,000 (4.8–28.2%) HCC cases annually around the world, with most of the cases occurring in the sub-Saharan African region where there is a higher incidence of HBV. Ethiopia has the highest prevalence of chronic HBV among African nations (6–7%), with approximately 11–288 HCC cases linked to chronic aflatoxin exposure and 21–643 HCC cases linked to the combined effects of AFs and HBV (Fazekas et al., 2005).Additionally, based on the AFs level (26 µg/kg) in basic cereals, Ayalew et al. (2018) calculated AFs exposure (1.4–36 ng/kg body weight/day). According to a cross-sectional study carried out in eastern Ethiopia, 33% of patients with CLD had a chronic HBV infection; nevertheless, the cause of the liver disease was unknown in over 50% of the patients (Zhao et al., 2018).

To ascertain the potential public health effects that can be linked to AFs exposure, epidemiological studies must be conducted in a region with a greater prevalence of AFs contamination (Hossain, 2003; Oberoi et al., 2005; Seenappa et al., 1980).Furthermore, with a death rate of 93.4 per 100,000, HCC is one of the leading causes of death in Ethiopia (Iqbal et al., 2010). Therefore, in order to limit AFs contamination, it is vital to determine whether exposure to AFs and CLD in Ethiopia may be related and to design suitable mitigation techniques.

Similar to *Aspergillus* mycotoxins, the prevalence of *Fusarium* mycotoxins has also been noted to be higher in Ethiopian commodities, causing higher health risks owing to dietary *Fusarium* mycotoxins exposure. Previous studies (Gambacorta et al.,2018; Seenappa 1980; Christensen et al.,1967; Candlish et al.,2001).

Have confirmed higher human exposure to *Fusarium* toxins in Ethiopia. Elevated *Fusarium* toxins exposure is known to cause serious health hazards both for humans and animals. According to the IARC, FB1 is possibly carcinogenic to humans (Group 2B) (Reddy et al.,2001). and many studies have reported a correlation between dietary FUMs exposure and esophageal cancer (EC) (Ikoma et al., 2015; Patel et al., 1996).A survey conducted in 2013–2016 on the incidences of EC across Oromia region in Ethiopia showed that two-thirds (68%) of EC cases (among 669 total EC cases in this region) originated from Arsi and Bale zones of Oromia Regional State, which are the main cereal producers in this region (Klieber, A. 2001). Similarly, another study also concluded that

consumption of hot cereal-based porridge had a positive link to higher EC prevalence in the area Hence, further epidemiological studies are required to determine the associations among consumption of hot cereal-based diet, FUMs exposure, and EC cases. Mycotoxins (e.g., AFs) exposure could exacerbate malnutrition, and a potential correlation between AFs exposure and childhood stunting has been reported (Asian et al 2012). Moreover, recently reduced rate of gestational weight gain was attributed to AFs exposure (Set and Erkmen,2014). Similar to HCC, childhood stunting is most prevalent in South and East Asia and sub-Saharan Africa, where foodborne AFs exposure is high (Iqbal et al., **2013).**

However, in Ethiopia, the synergy between mycotoxins exposure and malnutrition has not been well documented. Only one epidemiological study on AFs exposure among children (1–4 years of age) in Amhara and Tigray regions had been published, although the study findings did not show any associations between malnutrition in children and AFs exposure. Nevertheless, the presence of AFs (B2, G2, and M1) in 17% of the urine samples from children indicated that AFs exposure is a severe childhood health concern in Ethiopia (Yassihuyuk et al., 2014). Moreover, in Ethiopia, childhood malnutrition is higher, with 38% of children (<5 years of age) being stunted.(El_keday et al., 1995). . Therefore, there is an urgent need for more epidemiological studies to better understand the synergy between mycotoxins exposure and malnutrition as well as to implement regulatory measures to control AFs contamination in infant foods and maternal nutrition.

Capsicum products are used as seasoning and consumed in reduced portions. Consequently, there is no major food outbreak related to this spice. However, experimental research has already shown that pepper consumption may be a strong risk factor for gastric cancer. (López-Carrill et al. 1994) suggested that capsaicin, a compound present in pepper, behaves as a carcinogen. Conversely, consuming pepper tainted with mycotoxin on a daily basis can cause the body to accumulate toxic metabolites, which can result in acute intoxication and various mycotoxin-related illnesses. According to (Serra et al., 2002), red pepper's mycobiota may also increase the incidence of gallbladder cancer (GBC). (Ikoma and others, 2015). revealed that AFs and OTA had contaminated red peppers that were imported from Chile, Peru, and Bolivia. The authors report that all three countries' peppers had AF contamination below the EC-recommended threshold of

5 μg/kg, but the OTA contamination of two samples was above the EC recommended limit (15 μg/kg). The mean concentrations of OTA in the peppers from Chile (mean 355 μg/kg, range <5–1059 μg/kg) and Bolivia (mean 207 μg/kg, range 0.8–628 μg/kg), that have high incidence of GBC, were higher than that in Peru (14 μg/kg, range <5–47 μg/kg), which has an intermediate GBC incidence. Similarly, (Nogueira et al. 2015). Evaluated the plasma related to GBC cases in Chile. Authors found AFB1-adducts in 64% of the evaluated human blood samples (23 samples). In the study, the GBC cases were associated with the consumption of red pepper contaminated with AFB1. The results are not yet conclusive, but they point out the ingestion of mycotoxin-contaminated pepper as one of the determining factor for the development of GBC among the inhabitants of this country. A prolonged exposure to mycotoxin-contaminated red peppers may trigger different acute and chronic diseases. Specific medical research such as those developed by (López-Carrill et al., 1994) and (Serra et al., 2002) is needed, especially in communities with high consumption of this spice, such as the Andean, Mexican American, and Asian populations.

Many nations have put laws in place to restrict exposure to mycotoxins in trade; these laws are usually represented in parts per billion (ppb). Depending on the intended use, certain nations have varying restrictions; the strictest apply to exports and human consumption, while the highest apply to industrial goods. Due to the higher costs of satisfying the standards, such as testing, shipment rejection, and even eventual loss of admissibility into foreign markets, these regulations may result in lost trade income.

Further, mycotoxin contamination lowers product quality and reduces export values, which may lead to significant economic losses for producing countries. Mycotoxin contamination directly reduces availability of food and it has its own contribution to hunger and malnutrition (Udomkun et al., 2017a). According to WHO report, aflatoxin alone pose a significant economic burden through contamination of about 25% of the world food crops (WHO, 2018). Drought stress, failed to implement good agricultural practices (GAPs), poor postharvest practices and insect infestation are factors influencing mycotoxin contamination (*Paterson and Lima, 2010, Wagacha and Muthomi, 2008*). Further, socio-economic factors such as poor transportation and marketing systems, lack

of awareness, and insufficient regulations and legislations can also contribute to the risks of mycotoxin contamination *(Udomkun et al., 2017a*).

Table 3: Major common mycotoxins, their (established/evolving) and toxicities

Pepper Products	Mycotoxin	Country
Pepper fruits/Whole chilli/Pepper pod	AFs [a]; AOH [c]; AME [d]; CIT [e]; FB [g]; OTA [i]; ST [k]; TeA [l]	Argentina; Belgium; Chile; India; Nigeria; Pakistan; Sri Lanka; Turkey
Red pepper flakes	AFs; CIT; FB; OTA; ST	Belgium; Turkey
Chilli oil	AFs; OTA; ZEN [p]	United Kingdom
Paprika	AFs; OTA; ZEN	Australia; Brazil; Morocco; Peru; Portugal; Spain
Chilli/Ground chilli/Kashmiri chilli/Dried chilli	AFs; CIT; FB; OTA; ST; ZEN	Australia; Bolivia; Chile; India; Pakistan; Qatar; Saudi Arabia; Thailand Turkey; United States
Red pepper pastes	PAT [j]	Turkey
Red pepper powder/Ground red pepper/*Capsicum* powder	AFs; OTA; ST	Egypt; Ethiopia; Iran; Korea; Saudi Arabia; Turkey
Sweet peppers pod/Bell pepper pod/Fresh sweet peppers	AFs; ALT [b]; AME; AOH; FB; OTA; TeA [l]; TTX [m]; ZEN	China; India; Italy; Turkey
Dried sweet peppers	AFs; AME; AOH; DON [f]; FB; HT-2 [o]; NIV [h]; OTA; TeA; TTX; ZEN	Italy
Ground sweet pepper	AFs; ALT; AME; AOH; DON; FB; H-2 [n]; HT-2; NIV; OTA; TeA; TTX; ZEN	Italy
Fried sweet pepper	AFs; AOH; AME; DON; FB; H-2; HT-2; NIV; OTA; TeA; TTX; ZEN	Italy

Health risks by red peppers with mycotoxins

2.6 Mycotoxins Regulation in Ethiopia

The Food Medicine and Healthcare Administration and Control Authority (Ethiopian FDA) is primarily responsible for the Food and Drug Regulation and Control System in Ethiopia, which was established by Drug Administration and Control Proclamation No. 176/1999 and Council of Minister Regulation No. 299/2013. The Ministry of Health, Agriculture, Trade, Industry, Innovation, and Technology shares some of the responsibility. The Food Registration Directive was put into effect by the Ethiopian FDA in 2019, and it clearly identifies problems with food safety and contamination as well as the necessary actions based on the law. Moreover, a number of organizations, including

the commodities exchange agency and the standard agency. accountable to various ministries are also directly or indirectly involved in Ethiopian food legislation and regulation tasks. As a result, mandate overlapping and lack of coordination with regard to mycotoxins issues across these agencies are inevitable. Besides, most of the agencies do not consider mycotoxins level as a quality parameter or criterion for inspection and certification of local food establishments.

2.7. Raising Awareness and Adulteration Practices in Ethiopia

In Ethiopia, most people don't know much about molds and mycotoxins. Many Ethiopians are not even aware of the negative effects of eating moldy food, according to a study assessing knowledge on mycotoxins and their effects on health (Guchi et al., 2014). and that traders and farmers are mostly ignorant of the health hazards associated with prolonged exposure to AFs.

In general, majority of Ethiopians consider molds on food as harmless. Besides, it is believed that molds only affect the organoleptic properties of food, and can be easily treated by households cooking, drying ,or fermentation.(Beyene et al., 2016). performed a survey to identify Ethiopian mothers' knowledge and practice with respect to AFs contamination in complementary foods, and found that almost one-fourth of the respondents use moldy cereals to make local beverages such as *tela* (locally made beer) and *arake* (locally made spirit). Similarly, moldy *injera* (fermented flat bread mainly made of Teff) is usually dried under sun or heated to form *injera* crumb (*dirkosh*) (Worku and Fantahun, 2018). These traditional practices of food and beverage production could worsen AFs contamination among local people. Fraudulent traders in Ethiopia also frequently engage in deliberate adulteration and inadequate storage methods. According to Worku and Fantahun (2018), the most common practice among local traders is adding water to dry goods like peanuts and spices to make them heavier. This is dangerous because the tightly packed products allow mold to grow and produce mycotoxins. A training manual that was recently created by the Ethiopian Biotechnology Institute (EBTi) could aid in raising awareness along the crop value chain. It covers pre- and post-harvest practices that may have an impact on the growth of fungi that cause mycotoxin development in crops and offers mitigation technique

2.8 Mycotoxin Management Strategies

While there is no single and best mycotoxin intervention strategy to address mycotoxin contamination, multifaceted approaches at different stages in the value chains are encouraged. Udomkun et al., 2017). Pre- and post-harvest mitigation strategies, including good farming practice (GFP), good agricultural practice (GAP), and good handling practice (GHP) (Okoth,2016). Mainly used to prevent mycotoxins contamination are the most promising because they are cost-effective and easy to use by farmers. Besides, proper fertilization (Baholet et al.,2019). selection of disease-resistant breeding (Tucker et al.,2019). cropping rotation systems (Selvaraj et al., 2015) use of fungicides/pesticides, insects control measures, proper drying, appropriate storage, and transportation are encouraged. In addition, physical sorting methods, performed by removing damaged products based on quality parameters such as color, size, shape, density, and visible fungal growth Udomkun et al.,2017).are also known to have the potential to reduce the impacts of both mold infection and mycotoxins contamination. Moreover, the use of biocontrol agents has emerged as a promising approach. For example, the most successful biological control method to reduce AFs contamination is the application of non-aflatoxigenic *A. flavus* strains to soils, which competitively exclude naturally occurring toxin-producing *A. flavus* strains in the field, resulting in remarkable decrease in AFs production (Senghor et al., 2017). Some studies shows that during the processing adding some spices may decrease the mycotoxins level in the red pepper powder.

Cinnamon; mainly contains volatile compounds such as cinnamaldehyde (65%–80%) and eugenol (5%–10%) in the plant's bark (Rao and Gan,2014). These two compounds are responsible for their spicy sweetness and act as effective compounds against bacteria and moulds (Davidson and Naidu, 2000;Tajkarimi et al., 2010).Cinnamon has been used for cooking since ancient times. It is used in several cuisines as an aromatic condiment and flavouring additive. It also has great importance in medicine due to its antioxidant and antimicrobial properties (Nabavi et al., 2015). In the market, cinnamon can be found as whole or ground spices. The whole and ground cinnamon are widely used in Asian and Latin American countries, while the European and Mediterranean countries prefer the ground form Several studies had reported a low incidence of fungi in cinnamon samples.

In Indonesia, cinnamon recorded low fungal counts, with the most occurring species of Eurotium chevalieri (Nurtjahja et al., 2019).

Cloves; are dried flower buds of Syzygium aromaticum in the family of Myrtaceae (Raghavan, 2007). The name "clove" is derived from Latin clavus,

Spanish clavo, French clou, and German nelke, which means "nail" due to its resemblance to a nail's shape (Raghavan, 2007). The flower buds are picked before opening and sun-dried. After drying, the colour of the cloves turns dark brown with a slightly light brown content. Cloves have an intensely bitter and woody flavour and release a spicy-sweet aroma. The volatile oil of clove buds ranges from 5% to 20%, with eugenol, eugenyl acetate, and β-caryophyllene as the main components. The volatile oil has a significant amount of eugenol, which exhibits biological and antimicrobial properties (Leela and Sapna, 2008). In a recent study, Hlebová et al. (2021) revealed that clove oil has the highest inhibitory effect against all Aspergillus species tested, i.e., A. flavus, A. fumigatus, A. terreus, and A. niger (Hlebová et al., 2021). Eugenol volatile oils may inhibit the growth of microorganisms such as A. niger, Saccharomyces cerevisiae, Mycoderma sp.Lactobacillus acidophilus, and Bacillus cereus to varying degrees (Meena and Sethi, 1994; Leela and Sapna, 2008).

Fenugreek (*Trigonellafoenum-graecum* L., family—Fabaceae) is a leguminous herb grown across the globe, and more prominently in India and many Asian and North African countries.*(Wani and Kuma.,2018*).Traditionally, fenugreek has been used by Ayurvedic medicine practitioners for its medicinal properties. Furthermore, fenugreek has also been used in the home-based hair care remedies in India. Several studies have shown that fenugreek seed extracts possess antibacterial, antifungal, and antidandruff activity,[12,13] whereas variable results for antibacterial and antifungal activity have been reported for the fenugreek leaf extracts.[14–16] to date, no study has been carried out on the activity of fenugreek leaves extract against *Malassezia* spp.**Rues are** essential oil may have antifungal properties, but more research is needed to confirm its effectiveness and determine the specific types of fungi it can inhibits molds growth**Ginger** is well known for its antimicrobial properties and is applied as a natural solution to manage and avert

various plant pathogens *(Lei H, et al.,2017)*.therfore adding ginger is used to reduce Mycotoxins in the red pepper processing.

2.9 Reduction and Prevention of Mycotoxins

While there is no single and best mycotoxin intervention strategy to address mycotoxin contamination, multifaceted approaches at different stages in the value chains are encouraged. Pre-harvest and post-harvest mitigation strategies including good farming practices, good agricultural practices, and good handling practices are the most promising methods to prevent mycotoxins contaminations. Besides, proper fertilization, selection of disease-resistant varieties,cropping rotation systems, use of fungicides/pesticides, insects control measures, proper drying,and transportation are encouraged (Marshall et al., 2020). Systemic acquired resistance is potential to spray resistance inducers near harvest to prevent postharvest roots would benefit farmers by reducing inputs and lowering the risk of greater molds (Reglinski et al., 2023). Resistance inducers benefit plant health and protect the fruit by increasing quantities of antifungal compounds, phytoalexins, or other protective factors (Prusky and Romanazzi, 2023). Salicylic acid abscisic acid, and methyl jasmonates all act as resistance inducers of peppers. Phenol content and defense related enzymes are increased in fruits of all varieties (Liu et al., 2024). Which may have increased resistance levels. Spraying pepper plants 23 in the field with SA at 8 mM and citric acid at 30 mM reduced gray mold of peppers in storage and mycelial growth of the pathogen is sensitive to SA in vitro (Krasnow and Ziv, 2022).

When bell peppers were sprayed at 50 g/L with harpin protein, a known resistance activator, *B. cinerea* growth is reduced. Greenhouse grown fruits from plants regularly spray with harpin protein is higher soluble solids and acidity. These treatments can also extend the storage life of peppers under cool (7 oC) temperatures and modified atmosphere (Park et al., 2020). Therefore, resistance inducers likely increase the levels of certain antifungal compounds in the fruit. Heat treatment and UV-C application of post-harvested pepper prior to storage can directly inhibit pathogens and increase the resistance of the fruit. Treating red peppers with hot water at 55 oC for 12 sec (Fallik et al., 1999). Reduces decay and improves storability. Physical removal of spores and possible beneficial effects to the ' fruit's cuticle suggests having reduced disease. Heat

treatments from 45 to 55 oC is reduce spore germination and germ tube elongation. Using irradiation can reduce gray mold by killing pathogen spores and inhibiting latent infections. UVC light is germicidal to *B. cinerea* conidia and prevented gray mold of bell pepper. This treatment can also reduce damage from chilling injury and general decay from *Alternaria* spp. and *B. cinerea* (Krasnow and Ziv, 2022). More research is needed on this control strategy to determine practicality and economic feasibility for the pepper industry. Packaging and controlled atmosphere environmental regulation and temperature have a major effect on *Alternaria* spp.

This is a well-established and controlled agricultural settings, such as greenhouses, reducing relative humidity is known to limit gray mold during production. In storage, RH levels and atmospheric conditions are dependent on the crop being stored and the quantity. Disease severity levels are reduced when storage containers are kept at low O2 and high CO2 levels. The severity of gray mold of eggplant is reduced for fruits stored at 13 ℃ at 7.5% CO2 and 1.5% O2. Storage at 10 ℃ reduces the roots of pepper compared to 21 ℃.

Although cold storage helps to reduce fungal diseases, it will not eliminate B. cinerea, especially where24 crops are held for long periods. Peppers are typically washed after harvest, which can remove spores of B. cinerea but also contributes to high humidity in postharvest packaging. Typical packaging techniques for pepper may result in increased humidity during storage. Artificially reducing the humidity in packaging for peppers greatly reduced B. cinerea and other pathogens.

Additionally, modifying packaging atmosphere using moisture absorbance packets greatly increased the storage of green bell peppers by reducing the respiration levels of the fruit. Low temperatures in combination with modified atmosphere is important for maintaining high ascorbic acid (Krasnow and Ziv, 2022).

Physical management methods of cleaning and sorting of contaminated portions are most important steps of natural decontamination of mycotoxins to significantly reduced levels in diets with no risk of generating degradable toxic by-products (*Zhang et al., 2020*). Studies have showed that sorting and removing contaminated decaying fruits can

significantly reduce PAT levels in fruit products by up to 99% (*Luo et al., 2018*). as well as FBs and AFs (*Luo et al.,2018*).Soaking and washing grains in water can equally reduce mycotoxin levels. The mycotoxin contaminated fractions float due to their low density that can then be easily discarded. Washing has been reported to reduce ochratoxin A (OTA) and AFs in grains *(Adebo et al., 2021*). However, the use of such a physical method to manage mycotoxin contamination is laborious, inefficient, and often impractical, especially on a large scale (*Udomkun etal., 2017*).

Biological management methods through detoxification/degradation using microorganisms and enzymes have been reported as well as the use of herbal products and bio-adsorbents (Adebo et al., 2021). Probiotic bacteria and lactic acid bacteria (LAB) are effective detoxifying agents against mycotoxins due to their antifungal properties by primarily allowing for limiting fungal growth and hence toxin production (Nahle et al., 2022). In addition, fungal species of the genera*Aspergillus, Alternaria, Absidia, Armillariella, Candida, Dactylium, Mucor, Penicillium, Peniophora, Pleurotus, Trichosporon* and *Rhizopu*s play a key role in mycotoxin detoxification.25 In studies on mice and chicken, yeasts and their derived products were found to reduce AF by binding to AFB1 and the bound product eliminated via faeces (Adebo et al., 2021).

The limitation of using microorganisms for mycotoxin decontamination is the lack of information on the toxicity of derived by-products via degradation and the undesired effects of microorganisms that compromise the quality of food. Enzymes and metabolites of microorganisms also play a significant role in the detoxification of mycotoxins. Enzymes have catalytic potential to mitigate mycotoxin problems because of their specificity.

The mycotoxin detoxifying enzymes belong to oxidoreductase and hydrolase classes, some of which have been commercialized. (Lyagin and Efremenko, 2019).

Chemical management methods according to (Iha and Trucksess, 2019), the efficacy of washing white and black peppers with 18 agents with varying chemical qualities for minimizing AFs and OTA contamination Sulfuric acid, chloridric acid, phosphoric acid, benzoic acid, citric acid, and acetic acid were utilized as acidic compounds.

The detoxifying impact of chloridric acid ranged from 19.2 percent for AFB2 in white pepper to 33.7 percent for OTA in black pepper, whereas the other acids had a lower effect. The detoxification effect of alkaline substances (ammonia, sodium bicarbonate, sodium hydroxide, potassium hydroxide, and calcium hydroxide) was greater than that of acidic substances, with sodium hydroxide having a detoxification effect of 41.3 percent for AFG2 and55.3 percent for OTA in black and white pepper (*Iha and Trucksess, 2019*).

3. MATERIALS AND METHODS

3.1 Description of the study area

The study was conducted in households in three sub cities in Addis Ababa Addis Ababa is located at 9° I '48" north of equator and 38°44'24" east longitude with an area of540 km2 and2500 meter above sea level. The city was founded in 1886 and it has been serving as the capital city of Ethiopia since its foundation. According to the 20 I 0 census estimate the city had a population of about 3 million (CSA, 20 10). Its average temperature is 16 °c while its annual rainfall is 1221 millimeters. The city is divided into eleven subcities, and we have selected three of them, namely Lideta, Addis Ketema, and Kolfe Keranio.

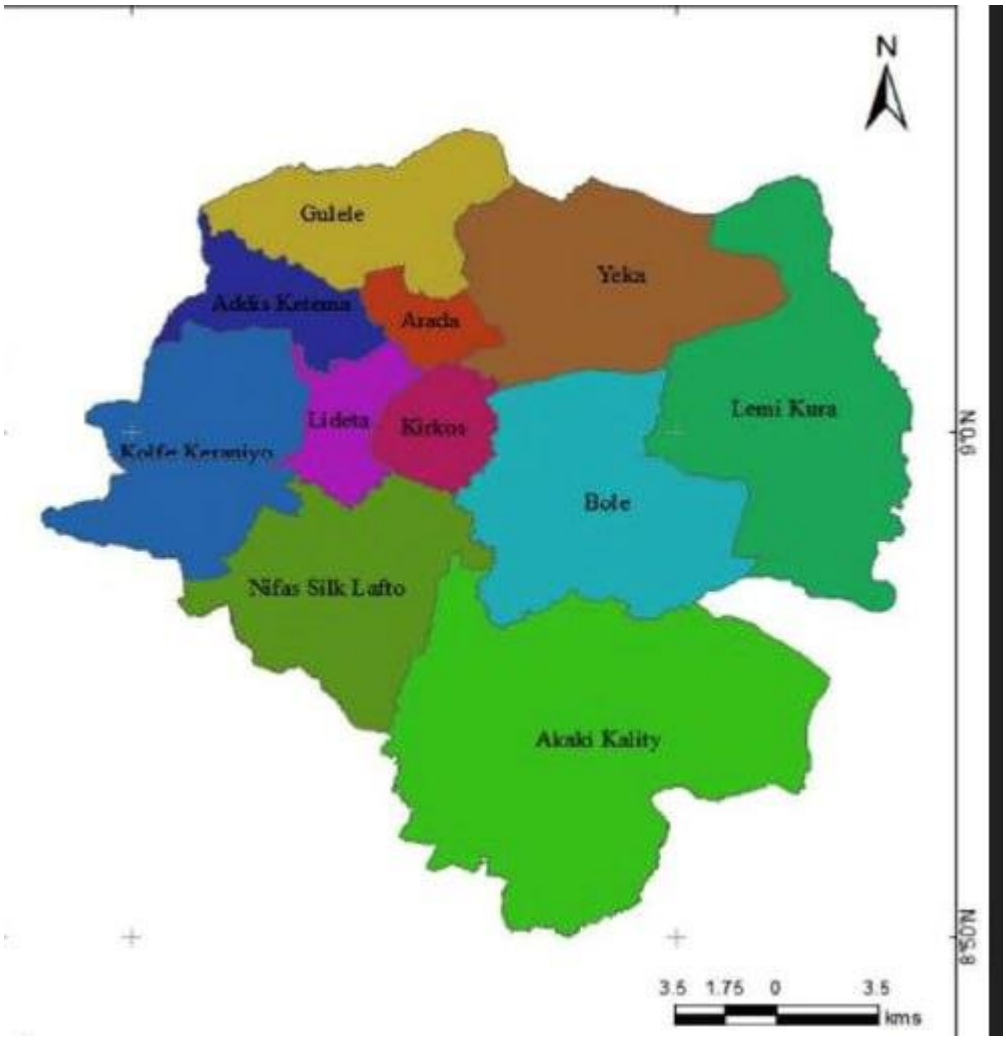


Figure 9: Map of Addis Ababa sub city where the red pepper samples were collected.

3.2 Sampling technique

Multistage sampling were used to collect red pepper powder samples at households in Addis Ababa. First three sub cities were randomly selected using lottery method from 11 sub cities and then after from each sub city one woreda was randomly selected.

3.3 Sample size determination

The red pepper powder sample size was calculated using a single population proportion formula used the prevalence of AFB1 in powder red pepper (92%) based on previous study (Hunduma et al.,2020).

$$n = \frac{\left(Z\frac{\alpha}{2}\right) 2 * P(1-P)}{d2}$$

Where;

N= the minimum sample size required for very large source population (>10,000).

$Z\frac{\alpha}{2}$=standard normal variable at 95% confidence level (1.96).

P= prevalence of AFB1 in red pepper powder reported in a previous study, which is 92%.

d= margin of error or degree of absolute precision /error tolerated (0.05).

therefore, our sample size were calculated by using the above formula and adding 10% non-response rate, then the final sample size were ;

$$n = \frac{(1.96)2 * 0.92(1-0.92)}{(0.05)2}$$

=3.8416*0.0736/0.0025

=113

113*10%=11

Hence the total sample size for the study was 113+11=124 (then it can distribute into the three sub cities.

3.4 The knowledge and practices survey

The knowledge and practices of the red pepper processors (women) from 124 households on issues related to mycotoxin were collected using structured questionnaires (Annex A). Data were collected only from three sub citiesThe questionnaire was initially prepared in English language and later translated into local language (Amharic).

3.5. Red pepper powder sample collection

Red pepper powder samples were collected from 124 households from randomly seleted three woredas from three sub cities (Kolfe Keraniyo, Addis Ketema and Lideta). The samples were collected in two phases: at the time of processing (December-January) and after six months of storage at households. In each household a maximum of 100 gm sample were collected using polyethylene bags and transported to the Center for Food Science and Nutrition research laboratory. Then after, a 40-composite red pepper powder samples (100 gm each) were prepared out of the 124 samples for each phase. Figure11. described how red pepper powder were prepared at households.

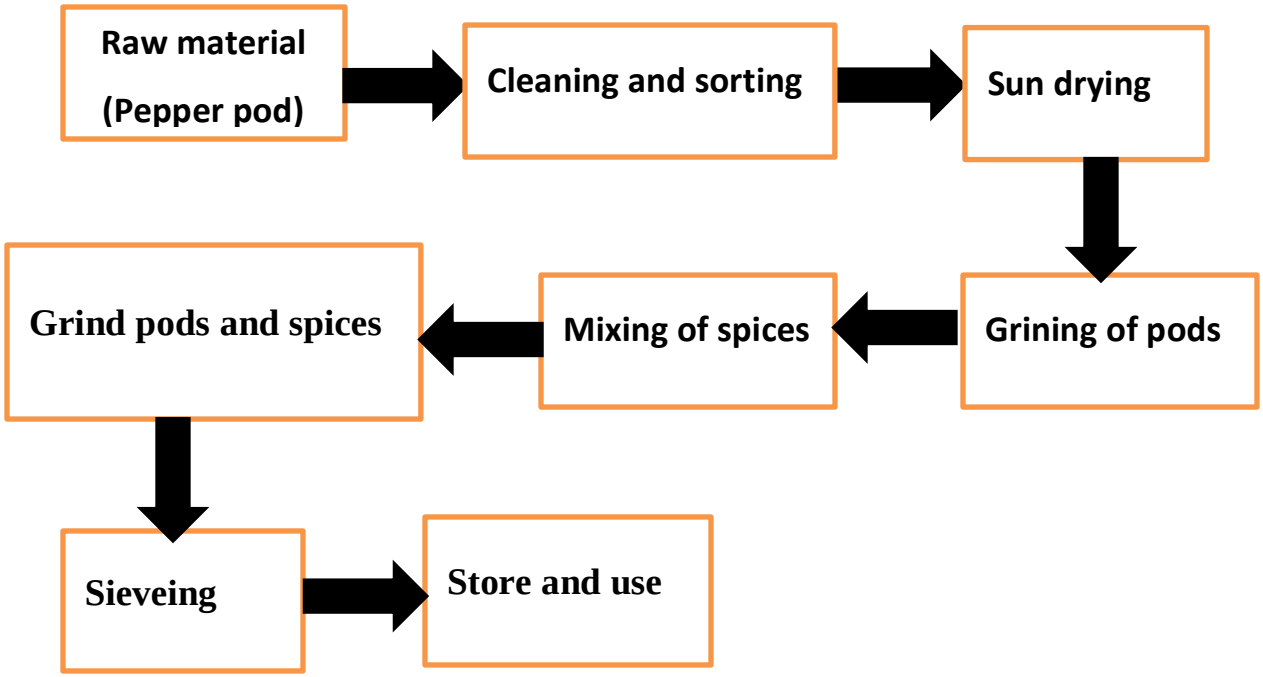


Figure 10: process flow diagram for red pepper powder preparation at households

The samples were collected from various sub-cities and woredas, which are shown in the (Table 4) below.

Table 4: Number of red pepper samples collected in each sub city in Addis Ababa for mycotoxin analysis

No	Name of sub city where the sample were collected	Name of woredas where the sample were collected	Number of samples collected
1	Kolfe keraniyo	Woreda 4	14
2	Addis ketema	Woreda 12	13
3	Lideta	Woreda 8	13
Total			40

3.6 Moisture analysis

The moisture content of all the samples were determined according to AOAC (2000), using the official method 925.10. A crucible was dried in an oven at 105^0C for 1hour and placed in desiccators to cool. The weight of crucible (W1) was determined. 5gm. Samples was weighed in the dry crucible (W2) and dried at 105^0C for 3 hours and after cooling to room temperature in desiccators it was again weighed (W3). The moisture content was determined by using. Eq.

$$\text{Moisture content in \%} = \frac{W2 - W3}{W2 - W1} * 100$$

Where

W1= weight of empty crucible

W2= weight of crucible and fresh sample

W3= weight of crucible and dry sample

3.7 Mycotoxins analysis

The levels of 26 mycotoxins (AFB1, AFB2, AFG1, AG2, α – Zearalenol, β – Zearalenol, zearalanone, zearalenone, Diacetoxyscirpenol, HT-2, T-2, 3-Acetyl deoxynivalenol, 15-Acetyl deoxynivalenol, deoxynivalenol (DON), Nivalenol, FB1, FB2, FB3, Fusaric acid, Moniliformin, Ocratoxin A (OTA), Beauvericin, Enniatin A, Enniatin A1, Enniatin B and Enniatin B1) in the red pepper powder samples were determined using an LC-MS/MS method (Ediage et al., 2015), validated according to the European Commission decision 2002/657/EC.

3.7.1 Chemicals and reagents

The chemical and reagent used for the mycotoxin analysis were formic acid, ammonium formate, methanol, acetonitrile (ACN), acetic acid, ultrapure water, and injection solvent: 30% acetonitrile, 70% water, containing 0,1% formic acid.

3.7.2 Sample preparation and clean-up, extraction, and filtration procedures

Extraction:

- The method is based on "dilute and shoot" principle. Two (2 g) of homogenized red pepper powder sample were weight into 50ml conical centrifuge tube and then extracted with 20 ml extraction solvent (80% ACN, 19% H_2O, 1% HCOOH). Samples were shake for 60 min on orbital shaker (250rpm)

Clean-up:

- Samples were centrifuged at 4200rpm for 5min.
- 1.2ml extract was passed across Captiva EMR - Lipid (Part No: 5190-1003).
- Defatted sample solution (450µl) was diluted with H_2O (750µl), mixed on Vortex and filtered across Nylon Syringe filter, pore size 0.22µm.
- Sample is ready for UHPLC/MS. The extracts are mixed with isotopically labelled internal standards (8) for each group of mycotoxins. The internal standard addition is performed in the needle of the autosampler, before injection onto UHPLC-MS/MS (Agilent Technologies 6460c MS/MS QQQ)

UHPLC-MS/MS system consist of:

- Analytical column Waters CORTECS UPLC® C18 2.1x100mm 1.6µm and guard column Waters CORTECS® UPLC C18 VanGuardTM 2.1x5mm 1.6µm guard column.
- Auto liquid sampler: Agilent Technologies 1290 series II with thermostat and column oven.
- Solvent delivery system: Agilent Technologies 1290 series II Flex pump
- Mass spectrometer: Agilent Technologies 6460c MS/MS QQQ with Jet Stream electrospray ion source.
- The MS/MS parameters were; Gas temperature 200^0C, gas flow 8 L/min, nebulizer 40 psi, sheath gas temperature 350^0C, sheath gas flow 11 L/min, capillary 3500 v and nozzle voltage of 500 v.

Solvent gradient conditions:

Mobile phase A: 0,1% formic acid, 5mM ammonium formate in H_2O. Mobile phase B: 0,1% formic acid, 5mM ammonium format in MeOH (75): ACN (25). Mobile phase C: 0,5% acetic acid in ultrapure water. Mobile phase D: Acetonitrile (ACN). Injection solvent: 30% acetonitrile, 70% water, containing 0.1% formic acid. The solvent gradient conditions at a flow rate of 0.3mL/ min are presented indicated in Table 5. The injection volume was 5µL and the column temperaturewas 50°C.

Table 5: Solvent gradient conditions for the UHPLC-MS/MS

Time [min]	**Mobil phase**				**Flow [ml/min]**
	A [%]	**B [%]**	**C [%]**	**D [%]**	
0.00	0	0	97	3	0.30
3.00	0	0	97	3	0.30
3.10	82	18	0	0	0.30
4.00	80	20	0	0	0.30
9.00	80	20	0	0	0.30
15.00	20	80	0	0	0.30
17.00	20	80	0	0	0.30
18.00	0	100	0	0	0.30
21.00	0	100	0	0	0.30

Table 6: The validation parameters for mycotoxin analysis: limit of detection (LOD) and limit of quantification (LOQ) for each mycotoxinss

Mycotoxin family	Mycotoxin	Abbreviation	LOQ; µg/kg	LOD; (µg/kg)
Aflatoxins	AFLATOXIN B1	AFB1	< 0.4	< 0.15
	AFLATOXIN B2	AFB2	< 0.4	< 0.15
	AFLATOXIN G1	AFG1	< 0.4	< 0.15
	AFLATOXIN G2	AFG2	< 0.4	< 0.15
Zearalenone + Metabolite	α – ZEARALENOL	α –ZEL	< 10	< 3
	β – ZEARALENOL	β –ZEL	< 10	< 3
	ZEARALANONE	ZAN	< 10	< 3
	ZEARALENONE	ZEN	< 5	< 1.5
A-Trichothecenes	DIACETOXYSCIRPENOL	DAS	< 3	< 1
	HT-2	HT-2	< 9.6	< 3
	T-2	T-2	< 9.6	< 3
B-Trichothecenes	3-ACETYL DEOXYNIVALENOL	3-ADON	< 20	< 7
	15-ACETYL DEOXYNIVALENOL	15-ADON	< 20	< 7
	DEOXYNIVALENOL	DON	< 20	< 7
	NIVALENOL	NIV	< 20	< 7
Fumonisins + Metabolite	FUMONISIN B1	FB1	< 25	< 8
	FUMONISIN B2	FB2	< 25	< 8
	FUMONISIN B3	FB3	< 25	< 8
	FUSARIC ACID	FA	< 3	< 1
	MONILIFORMIN	MON	< 3	< 1
Ochratoxin	OCHRATOXIN A	OTA	< 1.6	< 0.5
Emerging Mycotoxins	BEAUVERICIN	BEA	< 1	< 0.3
	ENNIATIN A	ENNI A	< 1	< 0.3
	ENNIATIN A1	ENNI A1	< 1	< 0.3
	ENNIATIN B	ENNI B	< 1	< 0.3
	ENNIATIN B1	ENNI B1	< 1	< 0.3

3.8 Data analysis

SPSS statistical software version 20 was used to analyse the data. Descriptive statistical approaches were used to analyse the survey data. Analysis of variance (one way ANOVA) was used to analyze the mycotoxins levels across woredas. Paired sample T-test were used to evaluate mycotoxin levels between phase baseline and endline. Significant differences were compared using Tukey method at P values <0.05.

3.9. Ethical clearance

Ethical approval was obtained from the College of Natural and Computational Sciences Institutional Review Board (IRB) of Addis Ababa University. The study's purpose and methods were thoroughly explained to red pepper processors at houseolds in Addis Ababa, and written consent were obtained. Prior to the survey, all questionnaires were translated into Amharic (the local language).

4 RESULTS AND DISCUSSION

4.1 Sociodemographic characteristics of the respondents

Of the 124 respondents involved in the study, 33.1% were from Addis Ketema and lideta sub city and 33.9% of them were from kolfe keranio sub city (Table 7). About three fourth of the respondents were above 40 years of age. Almost all (100%) of the respondents were females. Concerning the educational level of the respondents, 26.6% of them were illiterate, while 26.6% of the were completed teritary education. About 75% of the respondents were married, 45.2% of the were ortodox religeous follower, 66.9% of them were monthly income above 5000 Ethiopian Birr. Further, 33.1 % of them were housewifes in occupation.

Table 7: Sociodemographic information of the respondants, 2024

No	Sub city red pepper were collected	Frequency (N)	Percent (%)
1	Kolfe Keraniyo	42	33.8
	Addis ketema	41	33.1
	Lideta	41	33.1
2	Age of participants in year		
	>40	94	75.8
	<40	30	24.2
3	Educational background of the participant		
	Illitrate	33	26.6
	Can read and write	14	11.3
	Grade 1-8	25	20.2
	Grade 9-12	19	15.3
	Tertiary education	33	26.6
4	Marital status		
	Married	93	75.0
	Unmarried	24	19.4
	Divorced	7	5.6
5	Religious affilation of participant		

	Orthodox	56	45.2
	Musilim	30	24.2
	Catholic	22	17.7
	Protestant	18	12.9
6	Monthly in come of the participant in ETB		
	>5000	83	66.9
	<5000	41	33.1
7	Occupational status of the participant		
	House wife	41	33.1
	Marchant	36	29.0
	Government employee	24	19.4
	Private employee	23	18.5

4.2 Knowledge of the respondants related to mycotoxin contamination

An overview of the respondants knowledge related to mycotoxin contaminations are presented in Table 8. The majority (95.2%) of the respondents had no any knowlege about mycotoxins. Further, almost all were unaware of whether red pepper powder is contaminated with mycotoxins, its potential health impacts, or the consequences of the consequences of consuming red pepper powder contaminated with mycotoxins

Table 8. Respondants knowledge regarding mycotoxin contamination in red pepper powder, 2024

No	Factors	Frequency (N)	Percent (%)
1	Have you heard of mycotoxins (aflatoxins)?		
	• Yes	6	4.8
	• No	118	95.3
2	Have you heard that red pepper is contaminated with Mycotoxins/aflatoxin?		
	• Yes	124	100.0
	• No	0	0

3	If your answer is yes in Q. 2, when does contamination begin?		
	• In the field when growing	0	0
	• During harvest	0	0
	• After harvest	0	0
	• In-storage	0	0
	• Improper drying	0	0
	• Do not now	24	100.0
4	How do you know whether the red pepper is contaminated by Mycotoxins /aflatoxins (signs of contaminations)?		
	• Discolouration	0	0
	• Mouldiness and wetness	0	0
	• Presence of insects	0	0
	• Mouldy smell	0	0
	• Do not know	124	100.0
5	Do you think that consuming mycotoxins /aflatoxin contaminated red pepper powder has health impact?		
	• Yes	0	0
	• No	124	100.0
6	If your answer is yes for Q.5, what happened to the individuals that consume mycotoxin contaminated red pepper?		
	• Not applicable	124	100.0

4.3 Practices of the respondants related to mycotoxin contamination

The respondents' practices related to mycotoxin contamination in red pepper powder are presented in Table 9. All the respondants used the near by local market to collect the ripend red pepper pod used to process the red pepper powder. The respondants used their own quality parameters for selecting ripend red pepper pod, most (35.5%) of them used type while 25.8% of them used color as a selection criterion. All the respondents dry the ripend red pepper pod before processing, and used sun drying techniques using plastic sheets to reduce the moisture content of ripened red pepper pod before processing. This is a very ggood prsctice that needs to be encouraged. Reducing the moisture content (proper drying) of the ripened red pepper below 10-13% before processing is important in preventing and/or reducing mould spoilage and aflatoxin contamination along the production chain (Toontom et al. 2012; Sahar et al. 2015; Kabak and Dobson 2017; Costa et al. 2019; Tsehaynesh et al. 2021). Furthermore, all of them had the practice of

checking the moisture content of the dried red pepper pod before processing using traditional means, with their hands.

The respondants at households were also asked whether they were observed mold in the ripend red pepper pod used for processing. Unfortunately, all of the respondants identified mold in the ripended red pepper pod. Furthermore, thery were asked how to deal with a moldy red pepper pod, and they reported that they discard it. Regarding the processing steps, all respondents followed the necessary procedures to produce red pepper powder and incorporated various spices during processing. In terms of storage practices, all respondents used plastic jars for household storage, placing them in dry areas away from sunlight. The majority (42.7%) reported storing the product for up to 12 months

Table 9: Practices of respondents on issues related to mycotoxins in red pepper powder

No	Factors	Frequency (N)	Percent (%)
1	Where do you buy/collect ripened red pepper pod?		
	• From the nearby markets	124	100.0
	• Directly from the farmers land	0	0
2	What qualities do you look for selecting the ripened red pepper pod?		
	• Color	32	25.8
	• Type	44	35.5
	• Insect infestations	22	17.7
	• Odor	16	12.9
	• Free from mold infestation	10	8.1
3	Did you dry the ripened red pepper pod after collecting and/or receiving before processing?		
	• Yes	124	100.0
	• No	0	0
4	If your answer is yes for Q.3, how do you dry?		
	• Sun drying	124	100.0
	• Solar drying	0	0
5	If you use sun drying, where is the drying done?		
	• On the bare ground	0	0
	• Using plastic sheets on the ground	124	100.0
	• On the roof	0	0

6	After drying, did you check the moisture content of ripened red pepper pod?		
	• Yes	124	100.0
	• No	0	0
7	If your answer is yes for Q. 6, how did you check moisture content of ripened red pepper?		
	• Just by looking at them	0	0
	• By touching them by our finger	124	100.0
	• By indigenous knowledge (specify)	0	0
8	Did you observe mould in any ripened red pepper pod?		
	• Yes	124	100.0
	• No	0	0
9	If you're answer is yes for Q. 8, what did you do the mouldy ripened red pepper pod?		
	• Discard it	124	100.0
	• Sell it	0	0
	• Process it by mixing with pure one	0	0
10	What are the steps used to process red pepper powder at your home? ((multiple answers are possible)		
	• Collecting the ripened red pepper pod	124	100.0
	• Sorting, cleaning, remove stem of the red pepper	124	100.0
	• Meshing	124	100.0
	• Drying	124	100.0
	• Mixing different spices and salt	124	100.0
	• Ground to powder by grinding machine	124	100.0
	• Sieving	124	100.0
	• Packaging	124	100.0
	• Storage and use	124	100.0
11	What types of other spics or herbs do you add to process red pepper powder? (multiple answers are possible)		
	• Ginger	124	100.0
	• Garlic	124	100.0
	• Korerima (black cardamom)	124	100.0
	• Black & white cumin	124	100.0
	• Red onion (habesha)	124	100.0
	• Fenugreek	124	100.0
	• Rue	124	100.0
	• Rosemary	124	100.0
12	What types of packaging materials do you use to store the red pepper powder at your home?		
	• Plastic bag	0	0
	• Sacks	0	0
	• Plastic jars	124	100.0

13	How do you store the red pepper powder at your home?		
	• In a dry area with no reach of sun light	124	100.0
	• In a moist area	0	0
14	For how long do you store the red pepper powder at your home?		
	• For six months	17	13.7
	• For 12 months	53	42.7
	• For 12 months	54	43.5

4.4The occurrence of mycotoxin in red pepper powder

The level and occurrence of 26 mycotoxins were determined in the red pepper powder samples collected from households in three sub cities in Addis Ababa. Among the 26 mycotoxins, only 10 mycotoxins have levels above the limit of detection. so, we use the results of the 10 mycotoxins in this study. The red pepper powder samples were contaminated by different mycotoxins (AFB1, AFB2, AFG1 and AFG2, Diacetoxyscirpenol, ochratoxin A, Beauvericin, Enniatin A1, Enniatin B, and Enniatin B1) at varying occurrences and levels.

The occurrence and distribution of mycotoxins in red pepper powder samples were collected from three sub cuties in Addis Ababa were presented in Table 10. At the baseline mycotoxins were detected in all the samples at concentrations ranges from < LOD to 154 µg/kg. AFB1 was detected in all of the samples, with levels ranging from 1.70- 40.2µg/kg. In this study, 92.5% of the samples contained AFB1 above the European Commission's maximum limit of 5 µg/kg, and also 97.5% of the sample were above the level of 10 µg/kg for total aflatoxins (EU, 2006) and the Ethiopian standard (ET.std 16050). Further, AFB2 was detected in 97.5% of the red pepper samples with levels ranging from 0.69- 10.4 µg/kg. AFG1 was the principal component in all aflatoxin detected samples, all the 40 red pepper samples were highly contaminated by AFG1 with levels ranging from 6.9- 154 µg/kg. AFG2 was also detected in 85% of the red pepper samples with levels ranging from 1.0- 14.0 µg/kg. Diacetoxyscirpenol was detected in 2.5% samples with levels 8.44 µg/kg. Ochratoxin A was detected in 87.5% of the red pepper powder samples with levels ranging from 1.7-8.7 µg/kg, which was lower than the EU maximum limit, 15 µg/kg.

Furthermore, Beauvercin was detected in ll of the red pepper powder samples with levels ranging from 5.4-42.6 µg/kg. Enniatin A1 was detectd in 72.5% of the red pepper sample with levels ranging from 1.05-2.75 µg/kg. Enniatin B and Enniatin B1 were also detected inall of the red pepper powder samples with levels ranging from 6.1-28.4 µg/kg and 1.7-8.4 µg/kg respectively.

Table 10: The level of mycotoxins in red pepper powder samples collected from households in three sub cities in Addis Ababa at base line, 2024

No.	Sample code	Moisture content	Level of mycotoxins (µg/kg)									
			AFB1	AFB2	AFG1	AFG2	Diacetoxy Scirpenol	Ochra toxin A	Beau vericin	Enniatin A1	Enniatin B	Enniatin B1
1	AK1	5.3	20.4	4.18	142	4.0	< LOD	4.0	20.8	1.25	16.5	4.3
2	AK2	4.4	11.2	2.13	18.4	1.0	< LOD	4.9	7.41	1.22	14.9	4.2
3	AK3	5.0	10.1	2.84	29.4	2.0	< LOD	2.0	15.7	1.05	12.6	3.6
4	AK4	4.2	13.2	4.95	26.2	3.0	< LOD	3.0	16.9	1.30	10.2	3.30
5	AK5	5.25	7.28	1.07	31.5	2.0	< LOD	7.5	15.4	1.67	15.3	4.40
6	AK6	5.2	18.3	7.82	31.8	6.0	< LOD	1.80	12.8	< LOD	9.6	2.5
7	AK7	4.9	4.71	0.69	18.8	1.0	< LOD	4.2	9.51	1.52	21.0	5.1
8	AK8	2.9	5.80	1.56	19.3	1.0	< LOD	3.0	11.0	1.63	21.2	5.3
9	AK9	3.1	15.9	3.13	57.5	5.0	< LOD	< LOD	14.3	2.19	19.1	5.4
10	AK10	4.5	13.50	1.23	70.9	3.0	< LOD	1.70	14.4	2.75	20.7	6.30
11	AK11	4.0	12.2	2.38	49.5	2.0	< LOD	3.6	14.8	2.08	16.8	5.2
12	AK12	4.0	4.75	1.10	14.5	2.0	< LOD	2.6	9.65	< LOD	10.2	2.7
13	AK13	4.9	12.4	4.35	30.5	4.0	< LOD	2.60	16.2	1.12	12.5	3.10
14	KK1	5.5	1.70	< LOD	6.93	<LOD	< LOD	< LOD	19.8	1.24	13.5	4.10

15	KK2	4.4	7.23	0.90	32.4	4.0	< LOD	2.5	11.5	< LOD	9.3	2.5
16	KK3	5.9	7.38	0.88	19.6	< LOD	< LOD	8.7	11.6	< LOD	13.9	3.6
17	KK4	4.4	5.54	1.07	16.1	1.0	< LOD	2.0	10.2	< LOD	11.0	3.0
18	KK5	5.3	40.2	3.69	154	5.0	< LOD	16.1	20.4	1.67	17.5	4.9
19	KK6	5.4	9.41	1.60	28.3	1.0	< LOD	2.4	5.52	< LOD	10.1	3.1
20	KK7	4.0	5.60	1.54	18.2	< LOD	< LOD	2.3	5.40	< LOD	8.8	2.6
21	KK8	4.0	10.4	1.77	41.6	2.0	< LOD	< LOD	10.2	2.00	16.1	4.30
22	KK9	3.4	10.6	1.65	36.3	2.0	< LOD	2.4	13.1	1.60	13.0	3.80
23	KK10	3.9	15.4	1.63	43.8	1.0	< LOD	1.7	14.9	2.10	16.9	5.7
24	KK11	3.4	21.2	2.98	43.1	2.0	< LOD	6.1	16.8	1.95	20.1	5.7
25	KK12	4.3	6.42	1.30	22.8	< LOD	< LOD	1.7	18.6	2.27	21.1	6.1
26	KK13	3.4	6.97	1.57	9.28	< LOD	< LOD	< LOD	8.23	< LOD	6.1	1.7
27	KK14	3.7	19.3	3.79	46	6.0	< LOD	4.6	16.9	1.70	16.8	4.5
28	LK1	4.3	8.67	3.03	32.5	3.0	< LOD	4.3	11.3	1.19	18.0	4.6
29	LK2	4.9	5.28	0.83	17.5	1.0	< LOD	2.2	14.5	1.16	17.0	4.40
30	LK3	5.1	6.38	1.34	23.4	1.0	< LOD	2.2	12.3	1.03	15.9	4.2
31	LK4	5.0	5.79	0.70	15.4	1.0	< LOD	2.7	9.96	< LOD	19.5	3.30
32	LK5	5.0	9.41	2.24	30.6	2.0	< LOD	5.5	17.6	2.41	28.4	8.4
33	LK6	6.3	9.47	1.38	22.5	2.0	< LOD	5.4	20.4	1.98	26.8	5.5
34	LK7	4.3	7.40	1.29	10.1	< LOD	< LOD	< LOD	14.2	< LOD	8.6	2.3
35	LK8	5.0	13.8	1.93	104	4.0	< LOD	3.2	17.1	2.11	18.1	5.10
36	LK9	5.6	34.8	10.4	103	14.0	< LOD	4.8	14.1	2.60	23.9	6.2
37	LK10	5.9	16.0	4.43	44.5	5.0	< LOD	2.6	12.3	1.13	11.5	3.3
38	LK11	4.8	10.9	1.53	58.4	3.0	< LOD	7.4	42.6	2.37	24.1	7.8
39	LK12	6.0	8.23	1.22	16.2	1.0	8.44	3.5	11.3	< LOD	16.3	3.8
40	LK13	4.6	19.2	3.49	25.4	2.0	< LOD	3.7	10.1	1.67	18.7	5.8

* AK: Addis ketema, KK: Kolfe Keraniyo, LK: Lideta, AFB1:Aflatoxin B1, AFB2: Aflatoxin B2, AFG1: Aflatoxin G1, AFG2: Aflatoxin G2, LOD: Limit of detection

The occurrence and distribution of mycotoxins in powder red pepper samples stored at six months collected from three sub cuties in Addis Ababa are presented below in Table 11. Mycotoxins were detected with concentrations ranging from < LOD to 89.7 µg/kg. AFB1 was detected in all of the samples, with levels ranging from 1.03-23.5µg/kg. In this study, 75% of samples contains AFB1 aboove the EU limit, of 5 µg/kg, while 97.5% of the samples were above the EU limit of 10 µg/kg for total aflatoxins according to (EU, 2006). Further, AFB2 was detected in 70% of the red pepper samples with levels ranging from 0.51- 4.65 µg/kg. AFG1 and AFG2 were also detected in all and 95% of the red pepper powder samples with levels ranging from 3.89- 89.7 µg/kg and 0.5- 9.66 µg/kg respectively.

Further, Ochratoxin A was detected in all the samples with levels ranging from 2.1-18.1 µg/kg. Howevr, only 2.5% was above EU limit, 15 µg/kg. Similarly, Beauvercin was detected in all of the red pepper powder samples with levels ranging from 3.53-20.3 µg/kg. Enniatin B and Enniatin B1 were also detected in all of the red pepper powder samples with levels ranging from 4.86-21.3 µg/kg and 1.11-6.14 µg/kg respectively.

Table 11: The level of mycotoxins in red pepper powder samples collected from households in three sub cities in Addis Ababa after six months storage, 2024

No.	Sample code	Moisture content	Level of mycotoxins (µg/kg)									
			AFB1	AFB2	AFG1	AFG2	Diacetoxyscirpenol	Ochratoxin A	Beauvericin	Enniatin A1	Enniatin B	Enniatin B1
1	AK1	4.9	15.1	1.79	74	1.52	< LOD	4.58	11.5	3.64	21.3	6.14
2	AK2	5.2	16.6	4.07	47.6	4.99	< LOD	4.32	9.39	1.85	11.8	3.19
3	AK3	5.0	11.0	2.1	30.5	1.89	< LOD	4.46	11.6	< LOD	7.66	2.16
4	AK4	4.8	4.26	0.51	16.5	0.95	< LOD	5.89	6.81	< LOD	13.2	2.98
5	AK5	5.7	12.9	3.28	27.3	3.67	< LOD	4.04	11.2	1.19	10.8	2.71
6	AK6	5.1	7.57	< LOD	29.4	0.77	< LOD	6.45	10.1	< LOD	14.8	3.63
7	AK 7	4.5	4.11	< LOD	12	0.52	< LOD	4.8	7.88	< LOD	7.27	2.08
8	AK8	4.7	5.29	< LOD	15.7	0.5	< LOD	5.42	5.4	< LOD	13.1	3.44
9	AK9	5.1	8.27	1.14	21.5	<LOD	< LOD	4.47	5.75	< LOD	12.8	3.45
10	AK10	5.1	7.05	2.27	22.8	1.61	< LOD	8.27	9.24	< LOD	10.2	2.61
11	AK11	4.3	8.36	1.84	22.3	2.3	< LOD	4.62	7.51	< LOD	6.32	1.71
12	AK12	4.8	15.5	1.31	61.1	1.04	< LOD	6.22	17.4	< LOD	12.9	3.39
13	AK13	4.8	15.1	2.04	56.2	1.95	< LOD	5.01	16.5	< LOD	16.1	4.56
14	KK1	5.6	2.55	1.04	11.8	0.51	< LOD	5.51	12.7	< LOD	11.9	2.65
15	KK2	6.6	5.16	0.70	19.7	1.18	< LOD	4.64	11.4	< LOD	7.31	1.35
16	KK3	6.3	5.76	< LOD	12.4	0.61	< LOD	4.45	8.69	< LOD	8.2	1.95
17	KK4	6.3	8.69	< LOD	21.2	0.75	< LOD	3.21	3.79	< LOD	5.39	1.34
18	KK5	5.2	9.83	< LOD	20.1	1.2	< LOD	2.86	5.21	< LOD	6.15	2.01
19	KK6	5.5	5.86	1.19	15.4	1.41	< LOD	5.35	4.71	< LOD	6.04	1.12
20	KK7	4.2	7.62	2.26	28.4	1.32	< LOD	4.89	5.13	< LOD	7.21	1.94
21	KK8	6.2	9.4	1.24	40	0.52	< LOD	5.32	6.31	< LOD	9.97	2.66
22	KK9	5.0	6.79	< LOD	5.91	1.12	< LOD	2.1	5.78	< LOD	4.86	1.11
23	KK10	4.7	20.1	1.86	89.7	2.38	< LOD	7.39	6.63	< LOD	7.24	2.03

24	KK11	5.4	3.7	0.88	11.4	1.03	< LOD	4.33	4.37	< LOD	7.96	1.5
25	KK12	4.9	4.09	0.92	14.1	1.34	< LOD	4.49	5.86	< LOD	8.52	1.8
26	KK13	4.9	5.22	0.92	14.9	1.32	< LOD	5.84	4.81	< LOD	8.06	2.82
27	KK14	6.2	9.42	1.36	13.4	1.00	< LOD	8.54	3.53	< LOD	6.67	1.65
28	LK1	4.9	1.03	< LOD	3.89	1.45	< LOD	2.16	14.6	< LOD	8.86	2.22
29	LK2	4.8	3.63	< LOD	7.08	1.31	< LOD	4.08	12.3	< LOD	10.1	2.66
30	LK3	5.9	3.32	< LOD	7.46	0.54	< LOD	3	11.8	< LOD	8.84	1.8
31	LK4	5.3	11.1	0.96	13.6	1.63	< LOD	4.07	4.5	< LOD	7.52	1.74
32	LK5	4.9	4.55	0.67	23.5	0.79	< LOD	18.1	20.1	< LOD	12.1	3.19
33	LK6	4.7	5.29	< LOD	13	0.51	< LOD	9.83	11.27	< LOD	9.62	2.31
34	LK7	6.1	23.5	4.65	59.6	9.66	< LOD	4.97	9.07	< LOD	12.1	3.18
35	LK8	5.6	9.61	1.75	49.2	2.13	< LOD	8.55	10.9	< LOD	10.9	2.84
36	LK9	5.8	4.36	0.78	11.6	<LOD	< LOD	5.07	7.4	< LOD	6.76	1.41
37	LK10	5.9	17.3	1.41	78.2	2.24	< LOD	5.71	9.24	< LOD	9.12	1.91
38	LK11	5.2	8.51	< LOD	27.9	1.36	< LOD	3.91	14.4	< LOD	9.77	2.44
39	LK12	5.9	7.77	1.10	12.3	0.98	< LOD	5.54	6.68	< LOD	8.54	2.27
40	LK13	6.0	7.89	1.20	20.7	1.91	< LOD	3.72	12.3	< LOD	10.7	2.76

* AK: Addis ketema, KK: Kolfe Keraniyo, LK: Lideta, AFB1:Aflatoxin B1, AFB2: Aflatoxin B2, AFG1: Aflatoxin G1, AFG2: Aflatoxin G2, LOD: Limit of detection;

In this study, 92.5% of the red pepper powder samples at baseline (during processing) and 75% after six months storage was contaminated with AFB1 above the EU limit of 5 µg/kg, which is higher than in a previous study in Ethiopia by Aberedew and Ayelign (2022) who reported that 83% of red pepper powder samples collected from local markets in Addis Ababa were contaminated by AFB1 aboove the EU limit. Further, both at baseline and after six months of storage, 97.5% of the red pepper powder samples were above the EU limit, 10 µg/kg for total aflatoxins (EU, 2006) and the Ethiopian standard (ES ISO 16050). This result is higher than in a previous study in Ethiopia by Adugna et al. (2022) who reported that 75% of the red pepper samples collected from selected districts in Amahara region were contaminated by total aflatoxins above the EU limit. However, our study result was lower than a previous study in Ethiopia by Degefe and Geleta (2024) who reported that the total aflatoxin content in all packed pepper powder samples collected from supermarkets from North Shewa Zone, Oromia Region exceeded the EU limits.

In contrast to our results, low mycotoxin contamination of powder red pepper has been reported in other countries. A study by Set and Erkmen (2010) in Turkey reported that only 6.7% of packed red pepper samples collected was contaminated by AFB1 above the EU limit.

Compared to previous studies conducted in Ethiopia and other countries, the red pepper powder samples collected from households in Addis Ababa—particularly from the three sub-cities showed a higher occurrence of mycotoxins. This might be due to poor posthar vest practices such as insufficient drying, or slow drying and moisture re-introduction during storage/transporta tion are the major factors causing mould spoilage and aflatoxin contamination along the red pepper production chain (Hunduma et al. 2020; Kim et al. 2020).

The minimization of mycotoxin contamination is possible with good cultural practices of chillies (Reddy et al., 2001). Therefore, growing conditions, harvesting and processing methods, storage conditions and postharvestingtreatments should be carefully

controlled in order to prevent mycotoxin risks due to contaminated spices (Erdogan, 2004; Colak et al., 2006). In addition, packing is also a good measure to supply attractive and health products in hygienic condition (Erdogan, 2004). Data from Mandeel (2005) indicate that the packing method has a big effect on mould concentration and contamination level. Spices like red chili packed in gunny bags have higher colony counts than other methods (Mandeel, 2005). Further, there is a bad practice of spraying water in to the ripened red pepper so as to increase the weight along the value chain, even though the moisture content determined was below the recommended value, 13% (=0.50_{aw}) (Toontom et al., 2012). This can enhance the growth of molds are more developed on the red pepper can cause mycotoxins contaminations. Therefore, there must be continuous follow up will be needed to minimize mycotoxins during processing.

The levels of mycotoxins in red pepper powders and moisture content, collected from households in three sub cities in Addis Ababa at base line and after six months of strage are presented in Table 12 and 13 respectively. When we compare the levels of mycootoxins among subcities both at baseline and after six months of storage; at baseline, Enniatin B concentration showed statistically significant ($P< 0.05$) among sub cities. While after six months storage; the cooncentrations of Beauvericin, Enniatin B and Enniatin B1 showed statistically significant ($P< 0.05$) among sub cities. This may be attributed to the fact that the red pepper pods were sourced from various markets with differing mycotoxin concentrations, and that household across the different sub-cities use varied storage and processing methods.

Table 12: Mycotoxins levels in red pepper powders and moisture content, collected from households in three sub cities in Addis Ababa at base line, 2024

Sub cities	Number of samples	Moisture content	Level of Mycotoxins (mean) (µg/kg)								
			AFB1	AFB2	AFG1	AFG2	Ochratoxin A	Beauvericin	Enniatin A1	Enniatin B	Enniatin B1
Addis Ketema	13	4.43± 0.78a	11.51± 4.97a	2.87± 2.0a	41.56± 34.46a	2.76± 1.58a	3.41± 1.62a	13.76± 3.62a	1.61± 0.52a	15.43± 4.22ab	4.26± 1.16a
Kolfe Keraniyo	14	4.35± 0.84ab	11.95± 9.7a	1.87± 0.97a	37.02± 36.1a	2.66± 1.87a	4.59± 4.4a	13.08± 4.97a	1.81± 0.32a	13.87± 4.44a	3.97± 1.32a
Lideta	13	5.14± s0.63b	11.94± 7.9a	2.6± 2.58a	38.73± 31.5a	3.25± 3.62a	3.96± 1.58a	15.98± 8.57a	1.76± 0.6a	18.98± 5.67b	4.97± 1.76a

*Means with different letters across a column are significantly different ($P < 0.05$).

Table 13: Mycotoxins levels and moisture content in red pepper powders samples collected from households in three sub cites in Addis Ababa after six months storage, 2024

Sub Cities	Number of samples	Moisture content	Level of Mycotoxins (mean) (µg/kg)								
			AFB 1	AFB2	AFG1	AFG2	Ochra toxin A	Beauvericin	Enniatin A1	Enniatin B	Enniatin B1
Addis Ketema	13	4.92± 0.35a	10.08 ±4.52 a	2.04± 1.03a	33.61± 19.64a	1.81± 1.34a	5.27± 1.17a	10.02± 3.7b	--	12.17± 4.01b	3.23± 1.15b
Kolfe Keraniyo	14	5.50± 0.72b	7.44± 4.29a	1.24± 0.48a	22.74± 21.02a	1.12± 0.47a	4.92± 1.68a	6.35± 2.74a	---	7.53± 1.83a	1.85± 0.56a
Lideta	13	5.46± 0.51b	8.29± 6.19a	1.56± 1.29a	25.23± 22.96a	2.4± 2.46a	6.05± 4.18a	11.12± 3.99b	---	9.61± 1.59a	2.36± 0.55a

*Means with different letters across a column are significantly different (P < 0.05)..

The levels oof mycotoxins and moisture content in red pepper powders samples collected from households in three sub cites Addis Ababa before and after six months storage were compared the results are presented in Table 14.

Table 14: Mycotoxins levels and moisture content in red pepper powders samples collected from households in three sub cites Addis Ababa before and after six months storage, 2024

Type of Mycotoxins and Moisture content	Number of samples	Mean concentration at base line	Mean concentration at six months of storage	P value
Moisture content	40	4.63± 0.82a	5.3± 0.6b	<0.001
AFB1	40	11.81± 7.69b	8.57± 5.04a	0.02
AFB2	40	2.59± 1.97b	1.63± 1.0a	0.05
AFG1	40	39.05± 33.29b	27.08± 21.22a	0.03
AFG2	40	2.5± 1.52b	1.47± 0.94a	0.003
Ochratoxin A	40	3.96± 2.75a	5.43± 2.8b	0.04
Beauvericin	40	14.24± 6.04b	9.09± 4.0a	<0.001
Enniatin A1	40	1.38± 0.25a	2.23± 1.26a	0.41
Enniatin B	40	16.04± 5.16b	9.71± 3.25a	<0.001
Enniatin B1	40	4.39± 1.46b	2.46± 0.97a	<0.001

*Means with different letters across a row are significantly different ($P < 0.05$).

The moisture content mean increased significantly ($p < 0.001$) from 4.63% at baseline to 5.3% after six months stoorag. This might be due to the introduction of moisture from the environment during storage. The mean levels of aflatoxins AFB1, AFB2, AFG1, and AFG2, Beauvericin, Enniatin B and Enniatin B1 decreased significantly after six months of storage. This might be due to the fact that, some spices, like garlic, ginger, and cardamom, are known to have antioxidant and anti-fungal properties, which can potentially inhibit aflatoxin production or degradation. Mixing these spices with red pepper powder during production and then cooking the mixture with water may help detoxify aflatoxins. A study by Medalcho et al. (2023) in Ethiopia reported that mixing major spices to produce a spice mix red pepper powder had a positive effect on AFB1 detoxification in raw and cooked spice mix red pepper samples. However, in our study the mean level of ochratoxin A increased (from 3.96 to 5.43 µg/kg) significantly ($p=0.04$) after six months storage. This might be due to the difference mold species that contaminate the red pepper pod.

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

This study found that the respondents involved in red pepper processing at households in Addis Ababa do not have the knowledg about how the red peppers powder were contaminated by mycotoxins. Furthermore, they are unaware of the harmful effects that mycotoxin-contaminated red pepper can have on human health. The results of this study also confirm that, mycotoxins (such as AFB1, AFB2, AFG1, and AFG2, Ochratoxin A, Beauvericin, Enniatin A1, Enniatin B and Enniatin B1) widespread contaminants of powder red pepper produced at households in the Addis Ababa intended for human consumption. Nearly all of the levels of AFB1 and total aflatoxins detected in the red pepper samples were above the EU limit and the Ethiopian standard. However, only a small percentage of Ochratoxin A detected in red pepper powder samples exceeded the EU limit of 15 µg/kg. Furthermore, except Ochratoxin A, the mean levels of AFB1, AFB2, AFG1, and AFG2, Beauvericin, Enniatin B and Enniatin B1 decreased significantly after six months of storage.

5.2 Recommendation

In view of the present findings, the following recommendations are suggested:

- Awareness creation among the different stakeholdrers about the methods used to prevent red pepper powder from the risks of mycotoxin contamination such as good manufacturing and good storage practices and the human health impacts of mycotoxins.
- Further investigate the reasons behind the decrease in mycotoxin levels in red pepper powder after six months of storage.
- Recommended technologies for post harvesting like best drying ,storage and moisture content testing in farm

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Appindix 1

Annex A: Questionnaires to assess the knowledge and practice of the individuals involved in processing red pepper powder at households in Addis Ababa on issues related to mycotoxins

Information and Consent

Dear respondent,

Good morning/Good afternoon. Thank you for your interest to talk with me today.

I am _____ who is a member of the Center for Food Science and Nutrition of Addis Ababa University. We conduct a study to assess the knowledge and practice of red pepper powder processed at households in Addis Ababa, in terms of mycotoxins. Our goal is to improve the knowledge and practice of red pepper in terms of mycotoxins contamination. You are selected to take part of the study. If you are willing to take part of our study, you will be asked some questions on how to process red pepper powder in your home. In addition, you will also be asked to give us about 300 gm red pepper powder samples for checking its mycotoxins contamination.

We are, therefore, here to ask for your consent to take part of the study. You are totally free to accept or refuse to participate in the study. If you accept, we guarantee you that confidentiality of all information collected will be assured. You are free to quit at any moment of the survey, without any prior notice or justification. However, your honest participation will eventually help in assessing the knowledge and practices of red pepper powder in terms of mycotoxins contamination.

If you have any question, comments, or complaints, you can reach the researchers through the following address.

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Name of the participant for which consent is asked: _____Signature: _____

A1: Socio-demographic information

1. Questionnaire Code: _____ Location (Region): _____
2. District: _____ Sub-city: _____
3. Age of the participant in years:
    1. < 40
    2. ≥ 40
4. Participant's education level:
    1. Illiterate
    2. Can read and/or write (Informal education)
    3. Grade 1-8
    4. Grade 9- 12
    5. Tertiary education
5. Current marital status of the participant:
    1. Married
    2. Unmarried
    3. Divorced
6. Language spoken by the participants:
    1. Amharic
    2. Afan Oromo
    3. Tigrigna
    4. Other specify: _____
7. Religious affiliation of the participant:

1. Orthodox Christian
2. Muslim
3. Protestant
4. Catholic
5. Other, specify: _____

8. Monthly income (ETB):
   1. < 5000
   2. > 5000

9. Occupational status of the participant:
   1. Student
   2. Housewife
   3. House servant
   4. Daily laborer
   5. Merchant
   6. Government employee
   7. Private employee

A2: Knowledge of participants on issues related to mycotoxins in red pepper powder

10. Have you heard of mycotoxins/ aflatoxins?
    1. Yes
    2. No

11. Have you heard that red pepper is contaminated by mycotoxins/ aflatoxin?
    1. Yes
    2. No

12. If your answer is yes for Q. 11, when does contamination begins?
    1. In the field when growing
    2. During harvest
    3. After harvest
    4. In-storage

5. Improper drying
6. Other, specify _____

13. How do you know whether the red pepper is contaminated by mycotoxins/ afaltoxins (signs of contamination)?

1. Discolouration
2. Mouldiness and wetness
3. Presence of insects
4. Mouldy smell

14. Do you think that consuming mycotoxin/ aflatoxin contaminated red pepper has health impact?

1. Yes
2. No

15. If your answer is yes for Q 14, what happened to the individuals that consume mycotoxin/ aflatoxin contaminated red pepper?

1. Stunting in children
2. Liver cirrhosis (Liver cancer)
3. Immunity suppression
4. Death
5. Other, specify_____

A3: Practices of participants on issues related to mycotoxins in red pepper powder

16. Where do you buy/collect the ripened red pepper?

1. From the nearby markets
2. Directly from the farmers land
3. Other, specify: _____

17. What qualities do you look for selecting the ripened red pepper?

1. Color
2. Type
3. Insect infestations
4. Odor
5. Molds

6. Other, specify: _____

18. Did you dry the ripened red pepper after collecting and/or receiving before processing?

1. Yes
2. No

19. If your answer is yes for Q 18, how do you dry?

1. Sun drying
2. Solar drying
3. Other, specify_____

20. If you use sun drying, where is the drying done?

1. On the bare ground
2. Using plastic sheets on the ground
3. On the roof
4. Other specify: _____

21. After drying, did you check the moisture content of the ripened red pepper?

1. Yes
2. No

22. If your answer is yes for Q 21, how did you check the moisture content of the ripened red pepper?

1. Just by looking at them
2. By touching them
3. By indigenous knowledge (specify what)_____

23. Did you observe mold in any ripened red pepper? 1. Yes 2. No

24. If your answer is yes for Q 23, what did you do the moldy ripened red pepper?

1. Discard it
2. Sell it
3. Process it by mixing with pure one
4. Other specify: _____

25. What are the steps used to process red pepper powder at your home?

1. Collecting the ripened red pepper
2. Sorting, cleaning, remove stem of the red pepper

3. Meshing
4. Drying
5. Mixing different spices and salt

6. Ground to powder by grinding machine
7. Sieving
8. Packaging
9. Storage and use

26. What types of other spices/herbs do you add to process red pepper powder?

1. Ginger
2. Garlic
3. Korerima (black cardamom)
4. Black & white cumin
5. Red onion (habesha)
6. Fenugreek
7. Rue
8. Rosemary
9. Other, specify_____

27. What types of packaging materials do you use to store the red pepper powder?

1. Plastic bag
2. Sacks
3. Plastic jars
4. Others, specify_____

28. How do you store the red pepper powder?

1. Dry area with no reach of sun light
2. Moist area
3. Other specify_____

29. For how long do you store the red pepper powder at your home?

1. for six months
2. for 12 months
3. Over 12 months
4. Other specify _____

Appendix-2

Questionnaire for Aflatoxin Study translated in Amharic

አባሪ፡ በርበሬ በአዲስ አበባ በሚገኙ አባወራዎች ቀይ በርበሬ ዱቄት በቤት ውስጥ ለሚያዘጋጁ ግለሰቦች ከማይኮቶክሲን ጋር በተያያዙ ጉዳዮች ላይ ያላቸውን እውቀትና አሠራር ለመገምገም የተዘጋጀ መጠይቅ

ፈቃድ መጠየቂያ ቅፅ

ውድ ተሳታፊ፤

የመጣሁት ከአዲስ አበባ ዩኒቨርሲቲ የስነምግብና ኒውትሪሽን ትምህርት ማዕከል ነው:: በአሁኑ ጊዜ በአዲስ አበባ በቤት ውስጥ ሚያዘጋጁ የበርበሬ ውጤቶችን ጥራታችውን ከማይኮቶክሲን ንጥረ ነገር አንጻር ጥናት በማድረግ ላይ እንገኛለን፡፡ የጥናቱ አላማም በቤታቹ ዉስጥ የምታዘጋጁት በርበሬ ከማይኮቶክሲን ብክለት ነጻ ሁኖ እንዲዘጋጅ ለማድረግ ነው፡፡ በዚህም ጥናት ላይ እንዲሳተፉ እርስዎ ተመርጠዋል:: በዚህ ጥናት ላይ ለመሳተፍ ፍቃደኛ ከሆኑ፤በቤታቹህ ውስጥ በርበሬ እንዴት አድርጋቹህ እንደምታዘጋጁ ጥያቄዋችን ይጠየቃሉ:: ከዚህም በተጨማሪም 100ግራም የሚሆን የበርበሬ ናሙና ያስፈልገናል:: በመጨረሻም በርበሬውን ደህንነቱን ለማወቅ የማይኮቶክሲን ንጥረ ነገር ይዘት ምርመራ ይደረግበታል::

ስለዚህ እዚህ የተገኘሁት በጥናቱ እንዲሳተፉ ፈቃድ ለመጠየቅ ነው:: ጥናቱ ላይ መሳተፍም ያለመሳተፍም መብትዎ የተጠበቀ ነው:: በጥናቱ ላይ ከተሳተፉ የሚሰጡትን መረጃ ለማንም አሳልፈን የማንሰጥ መሆኑን ልናረጋግጥልዎት እንወዳለን:: ቃለመጠይቁን በማንኛውም ጊዜ ማቆም ከፈለጉ ማቆም ይችላሉ:: ነገረግን የሚሰጡን እውነተኛና የተሟላ መልስ የበርበሬ ደህንነትን ከማይኮቶክሲን ብክለት ለመከላከል ለምናደርገው ጥረት በእጅጉ ይረዳናል፡፡

ማንኛውም ጥያቄ እና ሐሳብ ውይም ቅሬታ ካላቹህ ቀጥሎ በተጠቀሰው አድራሻ ማድረስ ትችላላቹህ::

ፀጋዬ ቡናሮ የምግብ ሳይንስ እና ኒውትሪሽን ማዕከል፣ አዲስ አበባ ዩኒቨርሲቲ፡ አዲስ አበባ
ስቁ: 09- 74 -11- 16 57

ዶ/ር አበበ አየልኝ (አብሮ መርማሪ)፣ የምግብ ሳይንስ እና ስነ-ምግብ ማዕከል፣ AAU
ስልክ፡ 0911-105033; P.O.Box፡ 1176

ዶ/ር ጳውሎስ ጌታቸው (አብሮ መርማሪ)፣ የምግብ ሳይንስ እና ስነ-ምግብ ማዕከል፣ AAU
ስልክ፡ 09-89-82-11-71

በጥናቱ ለመሳተፍ ፍቃደኛ የሆነ (ች) ሰው ስም: _____ፊርማ: _____

ሀ 1: ግለሰባዊ መረጃ

1. መጠይቅ ኮድ፡ _____ አካባቢ (ክልል)፡ _____

2.ወረዳ፡_____ክፍለከተማ፡_____
___

3. የተሳታፊው ዕድሜ በዓመታት፡-
    1. < 40
    2. > 40
4. የተሳታፊ የትምህርት ደረጃ፡-
    1. ያልተማ ረ
    2. ማንበብ እና/ወይም መጻፍ ይችላል (መደበኛ ያልሆነ ትምህርት)
    3. ክፍል 1-8
    4. ከ9-12ኛ ክፍል
    5. የሶስተኛ ደረጃ ትምህርት
5. የተሳታፊው ወቅታዊ የጋብቻ ሁኔታ፡-
    1. ያገባ
    2. ያላገባ
    3. የተፋታ

6. በተሳታፊዎች የሚነገር ቋንቋ፡-

1. አማርኛ
2. አፋን ኦሮሞ
3. ትግርኛ
4. ሌላ ይግለጹ፡- _____

7. የተሳታፊው ሃይማኖታዊ ግንኙነት፡-

1. ኦርቶዶክስ ክርስቲያን
2. ሙስሊም
3. ፕሮቴስታንት
4. ካቶሊክ
5. ሌላ፣ ይግለጹ፡_____

8. ወርሃዊ ገቢ (ኢ.ቲ.ቢ.)

1. < 5000
2. > 5000

9. የተሳታፊው የስራ ሁኔታ፡-

1. ተማሪ
2. የቤት እመቤት
3. የቤት አገልጋይ .
4 የቀን ሰራተኛ
5. ነጋዴ
6 የመንግስት ሰራተኛ
7. የግል ሰራተኛ

A2: በቀይ በርበሬ ዱቄት ውስጥ ከማይኮቶክሲን ጋር በተያያዙ ጉዳዮች ላይ የተሳታፊዎችን እውቀት

10. ስለ ማይኮቶክሲን/አፍላቶክሲ

1. አዎ
2. አይ

11. ቀይ በርበሬ በማይኮቶክሲን/አፍላቶክሲን መበከሉን ሰምተዋል?

1. አዎ
2. አይ

12. ለጥያቄ 11 መልስዎ አዎ ከሆነ፣መበከል የሚጀምረው መቼ ነው?

1. በማደግ ላይ እያለ በመስክ ላይ
2. በመከር ወቅት
3. ከመከር በኋላ
4. በማከማቻ ውስጥ
5. ተገቢ ያልሆነ ማድረ
6. ሌላ፣ _____ ይግለጹ

13. ቀይ በርበሬ በማይኮቶክሲን/አፋልቶክሲን (የመበከል ምልክቶች) መበከሉን እንዴት ያውቃሉ?

1. ቀለም መቀየር
2. ሻጋታ እና እርጥበታማነት
3. የነፍሳት መኖር
4. የሻጋታ ሽታ

14. ማይኮቶክሲን/አፍላቶክሲን የተበከለ ቀይ በርበሬን መውሰድ በጤና ላይ ጉዳት አለው ብለው ያስባሉ?

1. አዎ
2. አይ

15. ለጥያቄ 14 መልስዎ አዎ ከሆነ፣ ማይኮቶክሲን/አፍላቶክሲን የተበከለ ቀይ በርበሬ የሚበሉ ግለሰቦች ምን ደረሰባቸው?

1. በልጆች ላይ የመቀነስ
2. የጉበት ካንሰር (የጉበት ካንሰር)
3. የበሽታ መከላከያ ማፈን
4. ሞት
5. ሌላ፣ ይግለጹ_____

A3: በቀይ በርበሬ ዱቄት ውስጥ ከማይኮቶክሲን ጋር በተያያዙ ጉዳዮች ላይ የተሳታፊዎች ልምዶች

16. የበሰለ ቀይ በርበሬ የት ይግዙ/የሚሰበስቡት? ss

1. በአቅራቢያ ካሉ ገበያዎች
2. በቀጥታ ከገበሬዎች

3. ሌላ፣ ይግለጹ፡_____

17. የበሰለ ቀይ በርበሬ ለመምረጥ ምን ዓይነት ባሕርያትን ይፈልጋሉ?

1. ቀለም
2. ዓይነት
3 . የነፍሳት መበከል
4. ሽታ
5. ሻጋታዎች
6. ሌላ፣ ይግለጹ፡_____

18. የበሰለውን ቀይ በርበሬ ከመሰብሰብዎ በፊት እና/ወይም ከተቀበሉ በኋላ አደርቀውታል?

1. አዎ
2. አይ

19. ለጥያቄ 18 መልስዎ አዎ ከሆነ፣ እንዴት ይደርቃሉ?

1. የፀሐይ ማድረቅ
2. የፀሐይ ማድረቅ
3. ሌላ፣ ይግለጹ_____

20. የፀሐይ ማድረቂያ ከተጠቀሙ, ማድረቂያው የት ነው የሚደረገው?

1. ባዶ መሬት ላይ
2. መሬት ላይ የፕላስቲክ ንጣፎችን መጠቀም
3. በጣሪያው ላይ
4. ሌላ ይግለጹ

21. ከደረቁ በኋላ, የበሰለ ቀይ በርበሬ የእርጥበት መጠን አረጋግጠዋል?

1. አዎ
2. አይ

22. ለጥያቄ 21 መልስዎ አዎ ከሆነ፣ የበሰለውን ቀይ በርበሬ የእርጥበት መጠን እንዴት አረጋግጠዋል?

1. እነሱን በማየት ብቻ
2. እነሱን በመንካት
3. በአገር በቀል እውቀት (ምን ይግለጹ)_____

23. በማንኛውም የበሰለ ቀይ በርበሬ ውስጥ ሻጋታን ተመልክተዋል?

1. አዎ

2. አይ

24. ለጥያቄ 23 መልስዎ አዎ ከሆነ፣ የሻገተውን ቀይ በርበሬ ምን አደረጉ?

1. ይጣሉት

2. ይሸጡት

3. ከንጹህ ጋር በመደባለቅ ያስኬዱት

4. ሌላ ይግለጹ፡

25. በቤትዎ ውስጥ ቀይ በርበሬ ዱቄትን ለማዘጋጀት ምን እርምጃዎች ጥቅም ላይ ይውላሉ?

1. የበሰለ ቀይ በርበሬ መሰብሰብ

2. መደርደር, ማጽዳት, የቀይ በርበሬን ግንድ ያስወግዱ

3. ማሽኮርመም

4. ማድረቅ

5 የተለያዩ ቅመማ ቅመሞችን እና ጨው መቀላቀል

6. መሬትን በዱቄት መፍጨት ማሽን

7. መንፋት

8. ማሸግ

9. ማከማቸት እና መጠቀም

26. ቀይ በርበሬ ዱቄትን ለማዘጋጀት ምን ዓይነት ቅመማ ቅመሞችን / ዕፅዋትን ይጨምራሉ?

1. ዝንጅብል

2. ነጭ ሽንኩርት

3. ኮረሪማ (ጥቁር ካርዲሞም)

4. ጥቁር እና ነጭ አዝሙድ

5. ቀይ ሽንኩርት (ሀበሻ)

6. አብሽ

7 ጤናዳም

8. ሮዝሜሪ

9. ሌላ፣ ይግለጹ

27. ቀይ በርበሬ ዱቄትን ለማከማቸት ምን ዓይነት የማሸጊያ እቃዎች ይጠቀማሉ?

1. የፕላስቲክ ቦርሳ

2. ቦርሳዎች

3. የፕላስቲክ ጠርሙሶች

4. ሌሎች፣ ይግለጹ

28. ቀይ በርበሬዱቄትን እንዴት ማከማቸት ይቻላል?

1. የፀሐይ ብርሃን በማይደረስበት ደረቅ ቦታ

2. እርጥበት አካባቢ

3. ሌላ ይግለጹ_

29. ቀይ በርበሬ ዱቄትን በቤትዎ ውስጥ ለምን ያህል ጊዜ ያከማቻሉ?

1. ለ ስድስት ወራት

2. ለ 12 ወራት

3. ከ 12 ወራት በላይ

4. ሌላ ይግለጹ

Appendix_3

i. ANOVA table for Mycotoxin levels at baseline

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
The moisture content of red pepper powder (RPP) samples samples at endline	Between Groups	2.746	2	1.373	4.428	.019
	Within Groups	11.474	37	.310		
	Total	14.220	39			
The level of AFB1 in the RPP samples at endline	Between Groups	48.628	2	24.314	.951	.396
	Within Groups	945.791	37	25.562		
	Total	994.419	39			
The level of AFB2 in the RPP samples at endline	Between Groups	3.213	2	1.606	1.722	.199
	Within Groups	23.317	25	.933		
	Total	26.530	27			
The level of AFG1 in the RPP samples at endline	Between Groups	861.549	2	430.774	.954	.395
	Within Groups	16710.771	37	451.642		
	Total	17572.319	39			
The level of AFG2 in the RPP samples at endline	Between Groups	6.058	2	3.029	1.183	.318
	Within Groups	89.636	35	2.561		

	Total	95.694	37			
The level of Ochratoxin A in the RPP samples at endline	Between Groups	8.967	2	4.483	.629	.539
	Within Groups	263.786	37	7.129		
	Total	272.752	39			
The level of Beauvericin in the RPP samples at endline	Between Groups	169.849	2	84.924	6.914	.003
	Within Groups	454.469	37	12.283		
	Total	624.317	39			
The level of Enniatin B in the RPP samples at endline	Between Groups	145.268	2	72.634	10.052	.000
	Within Groups	267.343	37	7.225		
	Total	412.612	39			
The level of Enniatin B1 in the RPP samples at endline	Between Groups	13.091	2	6.546	10.192	.000
	Within Groups	23.761	37	.642		
	Total	36.852	39			

ii. ANOVA table for Mycotoxin levels at endline\

ANOVA						
		Sum of Squares	Df	Mean Square	F	Sig.
The moisture content of red pepper powder (RPP) samples samples at endline	Between Groups	2.746	2	1.373	4.428	.019
	Within Groups	11.474	37	.310		
	Total	14.220	39			
The level of AFB1 in the RPP samples at endline	Between Groups	48.628	2	24.314	.951	.396
	Within Groups	945.791	37	25.562		
	Total	994.419	39			
The level of AFB2 in the RPP samples at endline	Between Groups	3.213	2	1.606	1.722	.199
	Within Groups	23.317	25	.933		
	Total	26.530	27			
The level of AFG1 in the RPP samples at endline	Between Groups	861.549	2	430.774	.954	.395
	Within Groups	16710.771	37	451.642		
	Total	17572.319	39			
The level of AFG2 in the RPP samples at endline	Between Groups	6.058	2	3.029	1.183	.318
	Within Groups	89.636	35	2.561		
	Total	95.694	37			

The level of Ochratoxin A in the RPP samples at endline	Between Groups	8.967	2	4.483	.629	.539
	Within Groups	263.786	37	7.129		
	Total	272.752	39			
The level of Beauvericin in the RPP samples at endline	Between Groups	169.849	2	84.924	6.914	.003
	Within Groups	454.469	37	12.283		
	Total	624.317	39			
The level of Enniatin B in the RPP samples at endline	Between Groups	145.268	2	72.634	10.052	.000
	Within Groups	267.343	37	7.225		
	Total	412.612	39			
The level of Enniatin B1 in the RPP samples at endline	Between Groups	13.091	2	6.546	10.192	.000
	Within Groups	23.761	37	.642		
	Total	36.852	39			

Appendix 4. Ethical clerance

COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES
Addis Ababa University
OFFICE OF THE DEAN
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አዲስ አበባ ዩኒቨርሲቲ

Ref.No. CNCSDO/734/16/2024
ቁጥር
Date April 02, 2024
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To Whom It May Concern

The College of Natural and Computational Sciences Institutional Review Board (**CNS-IRB**) Committee in its meeting held on **29/03/2024, Minute No IRB/02/2016/2024** has examined the project proposal entitled **"Mycotoxins contaminations in red pepper powder produced households in Addis Ababa (MSc)''** by **Tsegaye Bunaro** from the Addis Ababa University.

The proposal is approved for implementation effective from April 02, 2024.

With regards,

Tileye Feyissa, (Prof)
Dean, College of Natural & Computational Sciences
Addis Ababa University

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