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Dietary Practices, Nutritional status, Risk of exposure to aflatoxins and Pesticide among adolescents in Boarding - high schools in Kilimanjaro, Tanzania

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DIETARY PRACTICES, NUTRITIONAL STATUS, RISK OF EXPOSURE TO AFLATOXINS AND PESTICIDE AMONG ADOLESCENTS IN BOARDING - HIGH SCHOOLS IN KILIMANJARO, TANZANIA

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela Africa Institution of Science and Technology

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ABSTRACT

School feeding in low income countries relies mainly on cereals and legumes. Cereals are low in nutrients and its overdependence can lead to poor nutrient intake. Cereals and legumes are susceptible to aflatoxins contamination, causing both acute and chronic toxicity in human. The use of pesticide has been one of the measures to control aflatoxins. Inappropriate pesticides use may result to unacceptable residues in grains. A cross sectional study was conducted to assess dietary practices, nutrition status, the risks of exposure to aflatoxins and pesticide among adolescents through consumption of school meals in Kilimanjaro region. Food frequency questionnaires and 24 hours' dietary recalls were used to collect food consumption information. Nutrition status was assessed using anthropometry and test of hemoglobin levels. World Health Organization. Arthro plus and Nutri survey software were used to analyze anthropometry and dietary data respectively. Aflatoxin and pesticide residues were analyzed using High Performance Liquid chromatography (HPLC), and Gas Chromatography Mass spectrometer (GC-MS), respectively. Results shows that, maize based food and beans were consumed on daily basis with low intake of animal sources, vegetables and fruits. Mean intake of Vitamin C, iron, calcium and zinc were below the Recommended Daily Allowance (RDA). The average carbohydrates, fats and proteins intake were slightly higher than RDA for adolescents. Overall 23.1% of the adolescents were anemic, 25% overweight and 6.1% obese. Total aflatoxins contamination ranged from 0.20 - 438.53 µg/kg and aflatoxin B1 (AFB1) ranged from 0.44 µg/kg to 35.89 µg/kg. The highest exposure to total aflatoxins ranged from 0.70-973.45 ng/kg/bw/day and AFB1 ranged from 0.05-81.06 ng/kg/bw/day. Pesticide residues in all samples were below the detection limits, implying no risk to pesticide exposure among the studied individuals. Inadequate nutrients intake and the pronounced risk of exposure to aflatoxins could have been contributed by a monotonous cereal and legume based diet in boarding schools. The no detects of pesticide residues might have been contributed by degradation of pesticide due to the prolonged storage, milling process, and uses of silos for storage. The relevant ministries should consider food diversification and routine risk assessments of the susceptible crops throughout the value chain as a long-term intervention plan.

DECLARATION

I, Calista Nicholaus do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance for the dissertation entitled "Dietary Practices, Nutritional Status, Risk of Exposure to Aflatoxins and Pesticide among Adolescents in Boarding - High Schools in Kilimanjaro, Tanzania" in Partial Fulfilment of the Award of Doctorate of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

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LIST OF ABBREVIATIONS AND SYMBOLS

µg	Microgram
µL	Microliter
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFdB	African Development Bank
AFG1	Aflatoxin G 1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AFM2	Aflatoxin M2
AGRA	Alliance for Green Revolution in Africa
aOR	Adjusted Odd Ratio
ARfD	Acute Reference Dose
BDL	Below Detection limit
BMDL	Bench Mark Dose Lower limit
BMI	Body Mass Index
Bw	Body Weight
CAC	Codex Almentarius commission
CI	Confidence Interval
cm	Centimeter
cOR	Crude Odd Ratio
CRS	Catholic Relief Services
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethanes
dL	Deciliter
EAGC	Eastern Africa Grain Council

EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization
FFQ	Food Frequency Questionnaire
GC	Gas Chromatograph
GCLA	Government Chemist Laboratory Authority
GSHS	Global School Based Students Healthy Survey
H_2O	Water
Hb	Hemoglobin
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCH	Hexachlorocyclohexane
HCV	Hepatitis C virus
HPLC	High Performance Liquid Chromatograph
IARC	The International Agency on Cancer Research
IFRCCS	International Federation of Red Cross Crescent
IYC	Infants and Young Children
Kcal	Kilocalories
Kg	Kilogram
Km	Kilometer
LOD	Limit of Detection
LOQ	Limit of Quantification
mg	Milligram
mL	Milliliter
MOE	Margin of Exposure
MoEST	Ministry of Education Science and Technology

MoHCDGEC   Ministry of Health, Community Development, Gender, Elderly and Children

MOHSW   Ministry of Health and Social work

MRL   Maximum Residue Limit

MRM   Multiple Reaction Monitoring

MS   Mass Spectrometer

NATA   National Association of Testing Authorities

NatHREC   National Healthy Research sub -Committee

NBS   National Bureau of Statistics

NCD   Non- communicable diseases

ng   Nanogram

NIMR   National Institute for Medical Research

NM-AIST   Nelson Mandela African Institution for Science and Technology

PACA   The Partnership for Aflatoxin Control in Africa

r^2 Coefficient of correlation

RDA   Recommended Dietary Allowance

SD   Standard Deviation

SPE   Solid Phase Extraction

SPSS   Statistical Package for Social Sciences

sq   Square

SSA   Sub - Saharan Africa

SWeEt   Swedish Ethyl acetate

TBS   Tanzania Bureau of Standards

TFA   Trifluoroacetic Acid

TFNC   Tanzania Food and Nutrition Centre

TPRI   Tanzania Pesticides Research Institute

UN   United Nation

UNICEF   United Nation International Emergence Children Funds

URT   United Republic of Tanzania

US-EPA   United States - Environmental Protection Agency

WFP   World Food Programme

WHO   World Health Organization

χ^2 Chi square

CHAPTER ONE

INTRODUCTION

1.1   Background of the Problem

Adolescence is the age between 10 -19 years, which is a transition period from childhood to adult hood (World Health Organization [WHO], 2017). It is a period of rapid growth and development with high nutrients requirements (Das *et al*., 2018; Soman *et al*., 2017). It is estimated that 50% of adult weight, 45% bone mass and 20% of height are attained during adolescence (Melaku *et al.*, 2015; WHO, 2018).

Adolescents are characterized by high risk of malnutrition due to rapid growth and development (Gedefaw *et al*., 2015; WHO, 2018). Hence, adequate nutrition and safe diet is essential to cope with high demand of nutrients for physical growth, mental development, health and wellbeing during adolescents and later in adulthood (Christian & Smith, 2018; Das *et al*., 2017). Adequate nutrition, plays an essential role in preventing diet related chronic diseases like obesity, cardiovascular, various types of cancers and diabetes mellitus (Alkerwi *et al*., 2015; WHO, 2018). The need for balanced diet among adolescents has been widely researched and mainstreamed in nutrition intervention programs including school curricular however, its implementation is limited in low-income countries where food accessibility and nutrition knowledge has not been fully realized (Aliyar *et al.,* 2015; Erismann *et al*., 2016; Van Cauwenberghe *et al*., 2010). It is important to note that, a healthy life in adulthood is determined by the current health condition of adolescents hence, assessing their health and nutrition status at this age is important to reduce the burden of poor health, improve well-being and productivity in future and reduced chances of health risks to their children (WHO, 2018).

In developing countries of Sub Saharan Africa (SSA) school feeding programs have been mainly focusing on monotonous cereal and legume based diets in places where they are considered as staples and relatively cheaper and accessible (Ochola & Masibo, 2014; Ogum *et al.,* 2018; Worku *et al.*, 2017). The effect of monotonous cereal and legume- based diet is more pronounced in boarding schools where apprentices depend entirely on school meals. In Tanzania, school foods are obtained from own production, local farmers, markets or traders within or nearby regions. The estimated maize and beans consumption per person in Tanzania is 135 kg and 19.3 kg per year respectively, with nearly 400 g of maize consumed per day (Binagwa *et al.,* 2018; Mtaki, 2019). Maize based food constitutes more than 85% - 90% of

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the diet consumed for more than five days in a week (Mtaki, 2017). Cereal based meals are well documented to contain low key nutrients such as iron, zinc and calcium, even when eaten in combination with other food because the presence of anti-nutritional factors such as phytates inhibits uptake of these nutrients from the accompaniments into the body (Gibson *et al.*, 2010; WHO, 2018).

Besides cereals being low in nutrients, both cereals and legumes are highly susceptible to pests infestation particularly insects, which are the major cause of post-harvest losses in Sub-Sahara Africa (Babangida &Yong, 2011; Suleiman & Rosentrater, 2015). Apart from causing quantitative losses, pests infestation create a favourable condition for microorganisms' growth, particularly storage fungi *Aspergillus* species which are the main producers of aflatoxins (Manu *et al.*, 2019). These group of fungi are the common contaminant of cereals and oily seeds and may ultimately increase the risk of aflatoxins exposure in human and animals (Nyangi *et al.,* 2016).

Aflatoxins are toxic metabolites of the fungi especially, *Asperigillus flavus* and *A.parasiticus* (Medina *et al.*, 2014). Naturally, aflatoxin exist in more than twenty forms but the major four forms, Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2) are particularly dangerous to humans and animals health (The International Agency for Research on Cancer [IARC], 2015; Kumar *et al.*, 2017; WHO, 2018). Other natural toxins are Aflatoxin M1(AFM1), Aflatoxin M2 (AFM2 which are the hydroxylated metabolites of AFB1 and AFB2, respectively (IARC, 2012), which are excreted in milk and urine of animals including humans, following the consumption of AFBs contaminated feeds/food (Liu & Wu, 2010; Medina *et al.*, 2014; Okoth, 2016). Aflatoxin B1 (AFB1) and aflatoxin M1 (AFM1), are the most potent carcinogens among all mycotoxins and are classified as Group 1, meaning they have been proven to be carcinogenic to humans (IARC, 2012). Dietary exposure to aflatoxins, have been associated with pathogenesis of conditions such as primary liver cell carcinoma, immune suppression, growth retardation and acute aflatoxicosis (Wu *et al.*, 2011). Prevention of pest infestation and grain damage has been one of the leading strategies to control fungal contamination and aflatoxins formation both in the field and stored grains (Khan *et al.*, 2016). The use of synthetic pesticides has been the common technique used by farmers and grain dealers to control pests in grains (Adegbola *et al.*, 2012; Nukenine, 2010; Sexton, 2007). Kamala *et al.* (2018) indicated that stored maize, which were treated with synthetic insecticides, had low levels of aflatoxins compared to the untreated. However, inappropriate use of pesticides in developing countries has continued to be a major problem which has led to pesticide residues in food produces (Mahugija *et al*, 2017; Matowo *et al.,*

2020) with an ultimate serious risks to animal and human health (Kapeleka *et al.,* 2019; Sheikh *et al.,* 2011). Inability of farmers and grain handlers to adhere to the pesticide use instructions together with inadequate knowledge on pesticides toxicity, have aggravated the problem of pesticides malpractices in developing countries (Hajjar, 2012). Furthermore, there are no clear pest identification mechanisms hence, farmers often use either inappropriate pesticides or the correct pesticides are wrongly applied and in unsafe manner. Some farmers and grain handlers apply highly toxic chemicals in stored grains (Dahiru *et al.*, 2014; Grewal *et al.*, 2017; Mahugija *et al.,* 2017). All these together, contribute to serious problem of pesticide residues in the stored grains hence, increasing the risk of human exposure to these residues, through consumption of contaminated food (Sheikh *et al.,* 2011).

Therefore, this study sought to assess dietary practices, nutritional status, the risks of exposure to aflatoxins and pesticide residues through consumption of school meals among adolescents in boarding secondary schools in Kilimanjaro region Northern part of Tanzania.

1.2   Statement of the Problem

Despite global efforts of combating malnutrition, there are still large number of malnourished people in Tanzania and the Sub Saharan Africa at large (Mabhaudhi *et al.*, 2016; Tanzania Food and Nutrition Centre [TFNC], 2014). Generally, adolescents are vulnerable to malnutrition in its all forms but they are understudied hence underreported. Inadequate food intake, poor dietary diversification, poor nutrients bioavailability (because of the presence of inhibitors and the modes of preparation are threats to adolescent's nutrition status (Gebregyorgis *et al*., 2016; Melaku *et al.,* 2017). Also, adolescents are victims of the dietary shift from tradition diet to processed food together with sedentary lifestyle (Chinedu & Emiloju, 2012; Sarah & Amanda, 2015). Most nutrition interventions and healthy programs focus on under-five children and women of reproductive age particularly pregnant and lactating women leaving out the adolescents group who are as well vulnerable to malnutrition due to the additional nutrient demanded for physical growth and development (Christian & Smith, 2018; URT, 2016). For example in Tanzania adolescents girls belong to the group of women of reproductive age however, their nutrition wellbeing has been neglected, making them vulnerable to malnutrition and diseases (TFNC, 2014; URT, 2016).

Boarding schools are largely constituted of adolescents' boys and girls. Schools' adolescents in Sub Saharan Africa are served on monotonous cereal and legume based school meals which are inadequate in key nutrients and lacks diversity hence increase their vulnerability to malnutrition (Ochola & Masibo, 2014). Cereal based meals are low in key nutrients such as

iron, zinc and calcium and are high in anti-nutritional factors such as phytates (Gibson *et al.,* 2010; Miller & Welch, 2013). Phytates have inhibitory effects on the bioavailability of these nutrients which are essential for growth and development (Al Hasan *et al.*, 2016; Erkan, 2011; Resmi *et al.*, 2017).

Nevertheless, boarding school adolescents are particularly at high risk of malnutrition though this group has been largely understudied and therefore underreported. A study which was conducted among boarding secondary schools' adolescents aged between 13-19 years old in Nigeria indicated that 29% were underweight and 5.4% overweight and 2.4% obese (Omobuwa *et al.,* 2014). In Uganda, a study conducted in boarding school adolescents reported that 12.4% stunted, 2% thin, and 8% Overweight. Another findings were reported in Ethiopia whereby school adolescents aged 10-19 years old were 37.7% stunted and 21.1% underweight (Melaku *et al.,* 2015). Likewise, a study conducted in-schools' adolescents in private and government schools in Ethiopia showed that 11.2% were overweight (Berbada *et al.,* 2017).

Besides being low in nutrients, cereals and legumes are very susceptible to pest infestation at both pre-and post-harvest stages (Manu *et al.,* 2019; Suleiman & Rosentrater, 2015). Pests infestation damage grains, creating favorable environments and habitats for fungal contamination of agricultural produce (Danso *et al.,* 2018; Upadhyay *et al.*, 2011) thereby, increasing chances for mycotoxins production. Of the fungal genera, *Aspergillus* species, the main producer of aflatoxins which may increase the risk of aflatoxins exposure in human and animals if a routine consumption of these susceptible crops is allowed (Suleiman & Rosentrater, 2015). On the other hand, pesticide use has been considered a gold standard in management of pests infestation of crops during pre- and post-harvest (Adegbola *et al.,* 2012; Jallow *et al.,* 2017; Mahmood *et al.*, 2016). Nevertheless the excessive use of pesticides or misuse of highly toxic pesticides on grains has continued to be a public health concern in developing countries (Adegbola *et al.,* 2012; Anzene *et al.*, 2014; Grewal *et al.*, 2017). The use of highly toxic or over application of pesticide on grains is known to increase the risks of exposure to pesticides residues through consumption of the exposed grains (Amvrazi, 2011; Mahugija *et al.*, 2017).

However, in Tanzania there are limited information on boarding school adolescents' dietary practices and nutrition status. Likewise, the safety aspect of school meals has not given due attention. Therefore, this is the first study to document the dietary practices and safety of school meals among adolescents in boarding high schools in Tanzania.

## 1.3	Rationale of the Study

Adolescents in boarding schools depend entirely on school meals for their daily food intake while at school. Meals provided by schools are monotonous, mainly cereals and legumes based with low diversity and nutritionally inadequate. In addition, the safety of these foods (cereals and legumes) and nutritional status of the adolescents have not been monitored and documented. Adolescence period is characterized by rapid physical growth, change in body mass, hormones, and mental changes (Rahman *et al,* 2014). This makes nutritional requirements for the adolescents higher due to their demand for cognitive, physical growth, and development (UNICEF, 2011; Branca *et al,* 2015; Save the Children, 2015) as well as for better health in adulthood (Black *et al*., 2013; Venkaiah *et al.*, 2002). In Tanzania, little has been done to improve health and nutritional status of adolescents hence, scant data are available at national level which consider the nutrition status of adolescent girls only when grouped in women of reproductive age (FAO, 2008; Ministry of Health Zanzibar [MOH] *et al.*, 2016; URT, 2016; TFNC, 2014). Thus, few studies have focused on nutritional status of adolescents girls and boys (Cordeiro *et al.*, 2012; Maziya, 2014; Pangani, 2016; Teblick *et al*., 2017; Wilunda *et al.*, 2013). Further, there is limited information on the food safety aspects of school feeding including risks of exposure to aflatoxins and pesticide due to routine consumption of susceptible crops. Therefore, addressing health and nutrition needs of adolescents through school platform is an important step towards breaking the vicious cycle of intergenerational malnutrition. This created a need to conduct a study on assessment of dietary practices, nutrition status, the risks of exposure to aflatoxins and pesticide among adolescents in boarding high schools.

## 1.4	Research Objectives

### 1.4.1	General Objective

To assess dietary practices, nutrition status, the risks of exposure to aflatoxins and pesticide among adolescents in boarding high schools in Kilimanjaro region.

### 1.4.2	Specific Objectives

(i)	To assess dietary practices, knowledge and awareness on diet related diseases among adolescents in boarding high schools.

(ii)	To assess nutrition status of adolescents in boarding high schools.

(iii)   To determine the risk of exposure to aflatoxins through consumption of school meals among adolescents in the selected boarding high schools.

(iv)   To determine the risk of exposure to pesticide residues through consumption of school meals in the selected boarding high school.

1.5   Research Questions

(i)   What are the dietary practices among adolescents in selected boarding high schools of Kilimanjaro? Are the adolescents and staff responsible for school meals aware and knowledgeable on diet related diseases?

(ii)   What is the nutrition status of adolescents in selected boarding high schools?

(iii)   Does the consumption of school meals expose adolescents in selected boarding high schools to aflatoxin?

(iv)   Does the consumption of school meals expose adolescents in selected boarding high schools to pesticide residues?

1.6   Significance of the Study

This study determined dietary practices and nutrition status of boarding schools' adolescents and provided the prevalence of underweight, overweight, stunting and anemia. The study determined the nutrients intake among selected boarding school adolescents. This will provide an insight to policy makers, especially the Ministry of education and Ministry of health on proper nutrition interventions for adolescents to improve their health and reduce possible complications that might arise later in life. Through dietary practices assessment the study provided a benchmark for the Ministry of health in developing guidelines and standards on diets for adolescents for schools in Tanzania. The study established the levels of aflatoxins and pesticide contamination in school meals. In addition, the study provided baseline information for the safety assessment and monitoring of school feeding in Tanzania. This information is useful to the responsible ministries in the strategies to control food contaminants in Tanzania.

1.7   Delineation of the Study

This study was able to determine the dietary practices, nutrition status, and the risk of exposures to aflatoxins and pesticide among selected adolescents in boarding high schools, in

Kilimanjaro region. However, the interpretation of these findings should be done with caution due to some limitations such as being a cross section study was unable to show causal - effect relationship. The study used 24 hours' dietary recall and food frequency questionnaires to determine quantities of food consumed and habitual diet among adolescents respectively. These methods are subject to recall bias which might lead to over or under estimation of the food intake. However, these findings are confined to schools in one region from one agro ecological zone and limited to seasonal variability, therefore may not be generalized to the entire calendar year and other agro ecological zones of the country, due to the fact that, aflatoxins contamination of crops varies by season and location. Likewise, the pesticide residues in food was assessed just at one point of value chain and mainly in maize and legumes while the effects of pesticide residues might be distributed throughout the value chain.

CHAPTER TWO

LITERATURE REVIEW

2.1   Overview

Globally, there are more than 1.2 billion adolescents which accounts for 16% of the world population (Save the Children, 2015; UNICEF, 2012). Nearly, one fourth of the world adolescents are in sub-Saharan Africa (UNICEF, 2019). In Tanzania, there are more than 9 million adolescents, which are about 23% of the population (UNICEF, 2012).

Adolescence (10-19 years) is a period of rapid growth and development with biological psychological and emotional changes thus make the foundation of the current and future health (Das *et al.,* 2017; Ochola & Masibo, 2014). These changes contribute the increased nutritional demand on adolescents. However, this is a vulnerable period of life whereby unhealthy related behaviors begin and that may influence the onset of chronic disease (Das *et al*., 2018; Rathi *et al.*, 2017).

Adolescents are engaged in both health promoting and health risking behaviours (El Achhab *et al.*, 2016). Behaviors such as smoking, excessive alcohol and excessive dieting are considered as health risking as they contribute nutritional deficits at a time where the nutritional demand is higher in adolescents (El Achhab *et al.,* 2016; Rattay *et al.*, 2018). Moreover, health promoting behavior such as rigorous physical activities and sports increased demand for nutrients as well.

 Adolescents have received less attention in most of the health and nutrition intervention, research and programs in low and middle income countries (Lillie *et al.*, 2019; Korkalo, 2016; Maziya, 2014). Children under fives and women of reproductive age have been a priority. For example, in Tanzania the available nutrition data are in Demographic Health Survey reporting female adolescents aged 15-19 years who are considered as women of reproductive age and their data reports BMI and anemia only (MoHCDGEC *et al.,* 2016). Knowing the nutrition status of adolescents is very crucial because adolescences represent a window of opportunity to prepare for a healthy adulthood, and a stage where correction can be made to abate some nutrition problems in life and rebuild healthy eating habits and life style behaviors thus, preventing onset of nutrition related diseases late by Melaku *et al.* (2017).
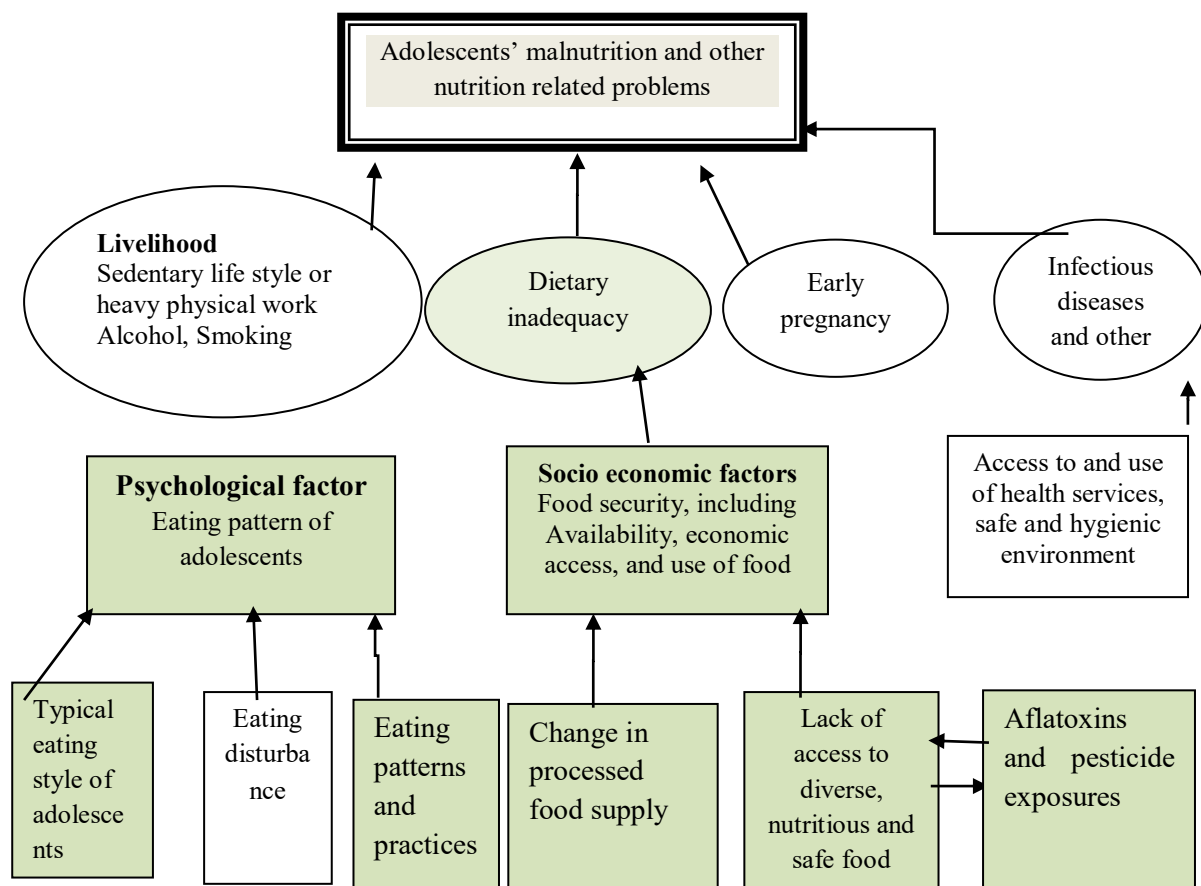
Figure 1: **Conceptual framework for adolescents' malnutrition and other nutrition related problems adopted and modified (WHO, 2005)**

2.2 Nutrition Status of Adolescents

Adolescents are regarded as healthier group, which receive less attention and care but they are vulnerable to all forms of malnutrition (Juwara *et al.*, 2016; Melaku *et al.*, 2017). Adolescents are characterized by unique lifestyle and eating behaviors that are important threats to their health (Keats *et al.*, 2017; Nasreddine *et al.*, 2014; Reicks *et al.*, 2015). Nutrition status of adolescents is highly influenced by dietary pattern which is contributed by food choices and lack of access to affordable and varied food sources, less income to purchase nutritious food, and ethnic or cultural influences on food preferences (Reicks *et al.,* 2015). However, nutrition deficits and poor eating habits established during adolescents have health consequences in the future life (Nti *et al.*, 2012; Rathi *et al.*, 2017). Therefore, adolescents' eating patterns are fundamental to their nutrition status. Likewise, nutrition status influences cognitive development and academic performances among school adolescents (Ochola & Masibo, 2014). School adolescents are prone to poor nutrition status due to their dietary patterns which is comprised of low diversity and nutrients inadequacies (Ochola & Masibo, 2014).

Under nutrition in school adolescents was reported in a study which was conducted among boarding secondary school adolescent girls aged between 15-18 years old in Nigeria which indicated that 57.1%, >50% and 4% were stunted, underweight and overweight, respectively (Anyika *et al.,*2009). Similarly, findings were reported in Ethiopia whereby school adolescents aged 10-19 years old, 37.7%, 21.1% were stunted and underweight, respectively (Melaku *et al.,* 2015). Likewise, in Bangladesh stunting and thinness in school adolescents were reported as 46% and 42.4%, respectively (Rahman *et al.,* 2014). Generally, underweight in adolescents has been reported in various regions of Asia and Sub-Saharan Africa to be ranging from 27% to 47% (UNICEF, 2011).

Another nutrition problem among adolescents is micronutrients deficiencies, this has been widely reported, whereby UNICEF estimated 40% anemia cases in ten countries worldwide are in the age bracket between 12-14 year, Moreover, data in 21 out of 41 countries indicated that more than one third of girls aged 15–19 are anemic (UNICEF, 2012). In a study conducted in Kenya and India, 31.4% and 55.8% of adolescent were anemic, respectively (Black *et al.*, 2013; Njura *et al. ,* 2013).

In Tanzania, prevalence of underweight (BMI<18.5) among adolescents' girls aged 15-19 years was 14.8% and overweight was 1.9% (MoHCDGEC, *et al*., 2019). Forty nine (49 %) were anemic (UNICEF, 2011). A study conducted in Dar-es-salaam and Pwani regions indicated that 14.2% of adolescents were stunted while 10.4% were underweight (Lillie *et al.*, 2019). On the other hand, The rate of over nutrition in adolescents is increasing globally (Bibiloni *et al.,* 2013; Fleming *et al.*, 2014; Lobstein, 2014; Seidell & Halberstadt, 2015). Approximately, more than 340 million children and adolescents aged 5-19 years were overweight and obese worldwide (WHO, 2016). In low income countries, the co-exist of overweight/obesity with underweight is more pronounced in urban areas due to the increase in sedentary life style and access to energy dense foods (Berbada *et al.*, 2017).

Changes in food habit and dietary patterns have become an emerging nutritional challenge worldwide (Soon & Tee, 2014; Wiggins & Keats, 2017). The trend of dietary shift from traditional diets to more processed food in conjunction with the increased sedentary lifestyle has increased in most of the developing countries (FAO, 2008; AGRA, 2019). Adolescents and young children are the most vulnerable for these changes (Chinedu & Emiloju, 2012; Sarah & Amanda, 2015). These changes could explain the increased rate of overweight/obesity and diet related diseases.

A study in Dar es salaam- Tanzania reported obese and overweight among school adolescents (8-13 years) were 6.7% and 15.9%, respectively (Pangani *et al.,* 2016). Moreover, another study conducted in secondary schools in Dar - es salaam found that adolescents 12-19 years were (13.9%) underweight (12.5%) overweight and (0.7%) obese (Mushengezi & Chillo, 2014). These expound the coexistence of double burden of malnutrition in adolescents. However, the consequences of poor nutrition during adolescence especially girls not only affects their body size but it may affects also children born to malnourished mothers (Branca *et al.*, 2015; Save the Children, 2015). It has been documented that provision of right quantity and quality of food to students improve nutrition status and academic performance (Sanya, 2015; WFP, 2019).

2.3 Adolescents Eating Habits

Adolescents are at great risks for malnutrition and nutrition related diseases due to unhealthy eating patterns (Nti *et al.*, 2012). Dietary habit of adolescents is influenced by peer, family, schools, community, mass media, body image perception and cultural and beliefs (Melaku *et al.*, 2017; The World Bank, 2017). Additionally, adolescents start to be more independent and begin to have access to money, make their own food choices and have access to food that are not available at home (Rodrigues *et al.,* 2017; The World Bank, 2017).

Adolescents frequently consume non - essential food which are high in fat, sugar and salts and lower in nutritional quality coupled with low intake of fruits, vegetables and animal sources food (Abizari & Ali, 2019; Tengia-Kessy & Killenga, 2020). These food are obtained from food restaurants, convenient stores and kiosks and sometimes from school cafeterias (Rathi *et al.,* 2017). These foods are considered palatable, attractive, cheaper and convenient. However, regular consumption of these food might result into negative health consequences including increased risks of malnutrition and chronic diet related disease (Al-Hazzaa *et al.*, 2012). Unhealthy school environment has negative influence on adolescents eating behavior (Smith *et al.*, 2013). Tengia-Kessy and Killenga (2020) found high proportion of school adolescents with unhealth dietary habits including high consumption of sweetened beverage, french fries and soft drinks in Kilimanjaro region .

Skipping at least one meal in a day and or replacing big meals with snack has been a common habit among adolescents (Rodrigues *et al*., 2017; El-Kassas & Ziade, 2017; Teferi *et al*., 2018). Breakfast was mostly skipped and rarely consumed by the adolescents (El-Kassas *et al.*, 2017; Nti *et al*., 2012; Rodrigues *et al*., 2017; Teferi *et al*., 2018). These habits has been attributed to inadequate dietary intake, consumption low quality diet and increased risks of

overweight and obesity (Mwaikambo *et al*., 2015). However, some study indicated that breakfast consumed by adolescents was just a plain cup of tea, millet porridge or left over food from the previous evening (Ochola & Masibo, 2014). Rodrigues *et al.* (2017) reported that only 47% 78% and 52% adolescents consumed breakfast, lunch and dinner daily, respectively. Meals skipping was associated with low consumption of fruits and vegetables among adolescents and subsequently was associated with higher intake of unhealthy food (Lazzeri *et al.*, 2013).

It is evident that dietary intake decline in quality from the childhood to early adolescents and intake of fruits, vegetable and milk declines during adolescence (Winpenny *et al.*, 2018). Inadequate intake of fruits and vegetable among adolescents has been reported widely in various studies (Deka *et al.*, 2015; Melaku *et al.*, 2017; Ochola & Masibo, 2014; Rathi *et al.*, 2017). In Ghana, 56% of adolescents rarely ate fruit and 48% rarely ate vegetables (Doku *et al.,* 2011). The data from the Global School-based Student Health Survey (GSHS), carried out among school adolescents in seven African countries, showed that 77.5% of adolescents did not meet the WHO daily-recommended intake of Fruits and vegetables (Peltzer & Pengpid, 2010). Low fruits consumption among adolescents' students was because provision of fresh fruits in schools was limited in most of the Secondary schools and it was difficult for adolescents to carry fruits from home to school.

Adolescents spent most of their time in schools. In schools especially in boarding schools, adolescents face limited food choices and resources that allow them to access nutritious food. They have access to snacks mostly starch, sugary and high salty snacks coupled with sweetened beverages stocked in the school shops or kiosks (Nguu-gutu *et al.,* 2014; United Nations, 2017). The regular consumption of these foods is associated with inadequate nutrients intake and increased risks to chronic diseases (Moreno *et al.*, 2010; WHO, 2018). The prospect of boarding scholars to meet their nutritional needs is mainly through school meals. Moreover, school offers opportunity to promote healthy eating and nutrition through formal learning and school feeding (Abizari *et al.*, 2014; Melaku *et al.,* 2017; United Nation 2017). It is access point to promote knowledge and awareness among parents and community members in prevention of nutrition related diseases (Letlape *et al.,* 2010).

2.4   Knowledge and Awareness on Diet Related Diseases

Nutrition awareness and knowledge can be important factors that may influence food choice and concomitantly, nutrient intake. The adequate knowledge in diet could indirectly contribute to the rate of morbidity and mortality of diet related chronic diseases among young people (Letlape *et al.,* 2010). Poor diets and wrong choice of food and consumption can be associated with the occurrence of diet related diseases, including cardiovascular disease, diabetes, cancer, osteoporosis and anemia (Kayode, 2016).

Adolescents are vulnerable to dietary changes and seem to adopt lifestyles behaviors that negatively affect their nutritional and health status and substantially increase their risk for developing diet-related diseases (Nti *et al.,* 2012). Adolescence save as a unique intervention point in life that offers a chance to acquire knowledge about nutrition that could prevent the onset of adult diet-related chronic illnesses (Nti *et al.*, 2012).

In a study conducted in school adolescents in Ghana found that adolescents had poor knowledge on diet related chronic diseases (diabetes, hypertension and obesity) (Nti *et al*., 2012). Moreover, a study conducted in South Africa indicated that 77% of the adolescents students had inadequate nutrition knowledge (Letlape *et al*., 2010). However, in a study conducted among school adolescents girls in Jimma Ethiopia among found majority (55%) of school adolescents girls a good knowledge in nutrition (Melaku *et al.,* 2017).

2.5   School Meals /Feeding

 School meals in developing countries of Sub Sahara Africa are comprised of mostly starchy staples that are locally available such as maize, rice, potatoes, plantains and wheat with less intake of fruits, vegetables and animal sources food (Best *et al*., 2010; Ochola & Masibo, 2014). School meals are meant to address nutrition and health problems of scholars (Thompson *et al.* 2013). The purposes of school meals differ between high income countries and low and middle income countries (Aliyar *et al.,* 2015). In higher income countries, school meals intends to tackle problems of over nutrition by ensuring that high quality food are offered to school children (Aliyar *et al.,* 2015; FAO & WHO, 2019). Meanwhile, in low and middle income countries, school meals are provided for the main purpose of alleviating hunger, act as safety net for poor households as well as to enhance school enrolment, students retention and to improve nutrition status (Aliyar *et al.*, 2015; Best *et al.,* 2010; FAO, 2013).

World Food Program has clearly demonstrated that school meals are effective way of improving academic performance, health and nutrition (WFP, 2013). Likewise, meals at schools are among key ways towards achieving the Sustainable Development goal 2: '*End hunger, achieve food security and improved nutrition and promote sustainable agriculture* (UN, 2019)*.* Therefore, provision of quality school meals is an excellent platform for interventions to improve health and nutrition status of adolescents (FAO & WHO, 2019).

In most developing countries including Tanzania, there are no guidelines and standards on the diet designed for school children/adolescents in relation to nutrients contents according to their nutritional requirements (Aliyar *et al.,* 2015; FAO & WHO, 2019; Roothaert *et al.,* 2021). Even in some policy documents school feeding is just mentioned without providing any details (Ministry of health and social welfare [MOHSW], 2015; URT, 2016). Moreover, there is no follow up on the safety of the school food due to low awareness and inadequate knowledge on safety issues as well as insufficient resources, lack of infrastructure and equipment (FAO & WHO, 2019; Roothaert *et al.*, 2021).

In Tanzania school, feeding is mandatory to all boarding schools but the coverage and implementation varies in different schools. The Ministry of Education, Science and technology (MoEST) coordinates school feeding in Tanzania. The Tanzanian government disburses funds to individual public boarding high school and school administrators procure food especially cereals and legumes form local traders/farmers through tenders. In ordinary level schools, parents contribute food stuff from their farm or in some cases they contribute money to enable the school to purchase food from local markets/farmers (Roothaert *et al.,* 2021). It is well documented that purchasing school food locally, provide small scale farmers with steady markets for their produces, increase their income and hence stimulate local economy and it is a better way to obtain fresh, unprocessed and diverse food (WFP, 2013). On the other hand, food from local traders/markets in rural areas of low income countries may not be fortified to enhance nutrients and usually not inspected hence, quality might be compromised (Wilson & Lewis, 2015; WFP, 2013).

Boarding scholars get three to four meals in a day such as breakfast, mid-morning, mid-day meal and supper (Roothaert *et al.*, 2021). Breakfast served in schools consist of cereals particularly thin porridge made of maize or and millet flour, or tea without milk and bread, while lunch and supper comprise mainly maize stiff porridge or dehulled maize and less often rice accompanied with beans or vegetables or meat stew (Abizari *et al.*, 2014; FAO & WHO, 2019; Lazzeri *et al.*, 2013; Roothaert *et al.*, 2021; Sanya, 2015). The school menus are highly

repetitive and monotonous cereals and legumes based diets. The monotonous diets especially those restricted on staples are inadequate in one or more micronutrients (Miller *et al.*, 2013). Moreover, cereals contain low content of nutrients such as iron, zinc, and calcium (Gibson *et al.*, 2010; Miller *et al.*, 2013). On the other hand, the bioavailability of these nutrients are inhibited by phytates which are nutrients inhibitor often found in whole legumes and cereals grains (Fanzo, 2012; WHO, 2018). Likewise, consumption of these high phytates food with other food compromise the bioavailability of minerals in those food (Gibson *et al.*, 2010). Phytates chelates metal ions zinc, iron and calcium and form complexes in the gastro intestine track which cannot be digested in humans due to lack of phytase enzymes (Gibson *et al.*, 2010). These nutrients are essential for growth and development during adolescence (WHO, 2018) but are often deficient in the developing countries and results into negative health consequences (Al Hasan *et al.,* 2016).

Apart from being low in nutrients, cereals and legumes are the most vulnerable to pests' infestation. During post-harvest the infestation is mainly due to inappropriate handling and management (Suleiman & Rosentrater, 2015; Tefera *et al.*, 2011). Cereals and legumes are normally stored to ensure constant supply and to preserve its quality for long term use (Jayas, 2012). However, subsistence farmers mainly store their grains for consumption purposes, seeds, and or for selling when price is favorable (Francisco *et al.,* 2009). The storage of grains especially maize take several months ranging between 5–12 months until the next harvesting season (Hell *et al.*, 2008; Manandhar *et al.*, 2018; Rugumamu, 2009). Maize and legumes are normally sundried then shelled manually thereafter stored in the traditional storage facilities which are placed in the kitchen, store room, or even in the bedroom (Stathers *et al.,* 2008; Suleiman *et al.*, 2017).

In Tanzania, Grains especially maize and legumes are stored in farmers' stores and storage periods may last for 8–10 months in a year (Mutungi, 2013). Customarily grains are stored in jute sacks, propylene sacks, or traditional cribs while modern storage techniques such as silos, hermetic bags and warehouses are mostly used for commercial purpose (Rugumamu, 2009). These traditional storage practices have no protection against major storage pests of staple food such as insects and molds (Tefera *et al.*, 2011).

During storage, pests and grain pathogens can cause substantial losses in quality and quantity, which is estimated at 15-25% of cereal grains and up to 100% legumes (Nukenine, 2010; Rugumamu, 2009). These losses might lead into food and nutrition insecurity and impact income generation by removing grains in the supply chain (Suleiman *et al.*, 2017; URT,

2019). Grain losses caused by insects and mycotoxins are among the highest in the post - harvest (Manandhar *et al*., 2018). Pests infestation through feeding and reproduction increase temperature and moisture contents of the stored grains hence, reducing the quality of the grains and lower the value of agricultural commodities (Dowell & Dowell, 2017). Additionally, the increase in moisture and temperature creates favorable environment and habitats for fungal contamination of agricultural produce (Suleiman & Rosentrater, 2015) thereby, increasing chances for mycotoxins production.

2.6   Fungi Contamination of Food

Mycotoxins is naturally occurring toxic produced by fungi *Asperigillus and Fusarium* species which contaminate food and feed stuff worldwide and cause significant health effects to human and animals (Logrieco *et al*., 2021). Also impact negatively the cereal trade both at domestic and in the international market (Kumar *et al.*, 2017; PACA, 2012; Phokane *et al*., 2019).

The   most important   mycotoxins of public health concern are aflatoxins, fumunisins, zearalenone, deoxynivalenol and ochratoxins and patulin (Assunção *et al*., 2018; Lee & Ryu, 2016). However, among other mycotoxins, aflatoxins and fumunisins occur mostly in staple grains especially maize (Kamala *et al.*, 2018; Kimanya *et al.*, 2014; Kumi *et al.,* 2014; Mwalway & Thole, 2016; Udomkun *et al.,* 2017). Aflatoxin is the most toxic that is produced by fungi *A flavus* and *A.parasiticus* which proliferate mainly in cereal staples such as maize, sorghum, rice, wheat, and edible nuts and legumes such as sunflower, sesame, cotton, groundnuts, beans and their products (IARC, 2015; Liu *et al*., 2010; Mmongoyo *et al*., 2017). However, the *A. flavus* is distributed worldwide but mainly in tropics and sub tropic areas where the climatic condition favor their growth (Catholic Relief Service [CRS], 2018).

The aflatoxins producing fungi occur at both pre-and post-harvest stages (IARC, 2015). At pre-harvest stage, fungal contamination is influenced by the interactions with the host plants, genotype, soil type and biological factors (Mannaa & Kim, 2017). At the post-harvest stage, fungal growth and development are determined by the substrate status (grains damage and nutrients contents), environmental factors (temperature, humidity, water activity, plant stress) and biotic factors (insects and microorganisms) (Mannaa & Kim, 2017). The extent of aflatoxins contamination also depends on geographical location, agriculture and agronomic practices as well as the susceptibility of the cultivars to fungal invasion during pre-harvest, storage, processing and transportation (Hell *et al*., 2008).

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Aflatoxins are grouped into more than twenty types but the major four common types are AFB1, AFB2, AFG1 and AFG2 which are known to cause adverse health effects in human and animals (Liu *et al.*, 2010). Aflatoxins B(B1 and B2) are produced by *Asperigillus flavus* while the *Asperigillus parasiticus* produced both aflatoxin B and G (Zuki-Orozco *et al.*, 2018). Additionally, AFM_1 and AFM_2 which are toxic metabolites of AFB1 and AFB2 that are found in urine, milk and milk products of the exposed animals and human (IARC, 2015; Lee *et al.,* 2016). The International Agency for cancer has classified the naturally occurring mixtures of aflatoxins as group 1 carcinogen to human (IARC, 2002). The AFB1 is the most toxic and occurs frequently with the highest concentrations (IARC, 2015).

2.7   Aflatoxins Contamination in Food Crops

It is estimated that 25% of the agricultural produce are contaminated by aflatoxins worldwide whereby maize and groundnuts are the most vulnerable crops (Logrieco *et al.*, 2021; Sharma *et al.*, 2018). Among other food crops, maize is cultivated and consumed as major staple food for millions of people in Sub-Saharan Africa (Kornher, 2018). Tanzania is currently ranked at the 19[th]position among maize producing countries worldwide (Mtaki, 2017). In sub-Saharan Africa maize is produced by resource-poor farmers in sub-optimal conditions and inadequate storage which favor fungal growth and subsequently aflatoxins production (Mutiga *et al.*, 2015; Wilson & Lewis, 2015). Maize is a good substrate for fungal growth and toxin production as it provides source of energy in form of carbohydrates (Atanda *et al.*, 2013).

In 2004-2005 aflatoxicosis outbreak in Kenya, it was reported that home grown maize from subsistence farmers was the source of the contamination (Yard *et al.*, 2013). The aflatoxins contamination of homegrown maize in Kenya was significantly higher up to 48 000 µg/kg and 24 000 µg/kg in 2005 and 2006, respectively while in 2007 contamination was up to 2500 µg/kg (Daniel *et al.*, 2011). In another study conducted in Makueni and Kibwezi districts in Kenya, aflatoxins contamination of maize from households ranged between 18 and 480 µg/kg (Kilonzo *et al.*, 2014). A study in Burundi and Eastern Congo assessed aflatoxins in maize grains and maize flour obtained from local markets. Contamination in whole maize ranged between 2.7- 330 µg/kg and 2.2-73.2 µg/kg, respectively while in maize flour contamination range was between 3.2-350 µg/kg and 2.5-320 µg/kg in Burundi and Eastern Congo, respectively (Udomkun *et al.,* 2018). In Malawi aflatoxins contamination in maize from household was up to 140 µg/kg (Mwalwayo & Thole, 2016).

Aflatoxins contamination and sometimes in conjunctions with other mycotoxins such as fumonisin have been reported in maize and maize products from different agro ecological zones in Tanzania (Kamala *et al*., 2015; Kimanya *et al*., 2014; Magoha *et al*., 2016). Makori *et al*. (2018) reported aflatoxins total ranged between 56.8-427.8 µg/kg in maize used for complementary food in Dodoma municipality and Chamwino districts in Dodoma region. High aflatoxins contamination ranged between 10-51, 100 µg/kg in home grown maize was reported during aflatoxicosis in Manyara and Dodoma in Tanzania in 2016 (Kamala *et al.,* 2018). In Rombo district Northern Tanzania Aflatoxins contamination in maize flour for complementary food was ranged between 0.11- 386 µg/kg (Kimanya *et al.*, 2014).

Morever, rice is among cereals that are suitable substrate for growth of mycotoxigenic fungi especially when processing and storage conditions are sub-optimal (Elzupir *et al.*, 2015). In Tanzania rice is ranked the second most important food crop after maize, it is widely produced and consumed in the country mostly in urban (URT, 2019). About 30% of the rice produced in the country is consumed by subsistence farmers households, whereas the remainder is sold in the domestic and regional market (Wilson, 2019). In the local markets, rice is mostly sold unpacked and stored in polypropylene bags/sacks which increase accumulation of fungi and hence toxins production (Lee & Ryu, 2016; Wilson, 2019). The level of aflatoxins contamination reported in rice is lower compared to many other cereals. Aflatoxins contamination in rice reported in Nigeria ranged between 4.1-309 µg/kg (Makun *et al.,* 2011). In three different agro ecological zones of Benin, Cameroon and Senegal the level of aflatoxins contamination in raw rice ranged from 0.1 to 45 µg/kg (Tang *et al.*, 2019). Another study which was conducted in ten countries of Africa reported highest aflatoxins contamination in rice up to 1642 µg/kg (Lee & Ryu, 2016).

Kimanya *et al.* (2016) reported low aflatoxins contamination in rice from Kilosa, Mbarari, and Misungwi districts located in Eastern, Southern highlands and Western agro ecological zones in Tanzania, respectively. In this study, 15% of samples had detectable levels ranged between 0.01-3.83 µg/kg (mean 1.19 µg/kg) (Kimanya *et al.*, 2016). Nevertheless, the prolonged consumption of low levels of aflatoxins contamination is also a concern as it may result into chronic exposures leading to adverse health effects (Elzupir *et al.,* 2015, 2017).

On the other hand, beans are recognized as good sources of proteins and livelihood for low income earners (Hayat *et al.,* 2014). Beans are highly consumed together with cereals as an accompaniment or cooked together as part of the mixed meal. In Tanzania, the average consumption of common beans per person per year is estimated 19.3 kg and contribute about

16.9% protein and 7.3 calories (Binagwa *et al.,* 2018). Despite being highly consumed, beans are rarely assessed for aflatoxins contamination. The rare and low aflatoxins contamination in beans have been implicated by the presence of phenolic compounds that have synergistic effects in the growth and development of toxigenic fungi (Mutegi *et al.,* 2018). In Babati district aflatoxins contamination was reported in beans ranging between 2-14.2 µg/kg (Nyangi *et al*, 2016). Moreover, a study in Burundi and Eastern Congo found aflatoxins contamination in beans was ranged between 2.5-6.6 µg/kg and 1.9-6.4 µg/kg, respectively (Udomkun *et al*., 2018). High aflatoxins contamination in beans ranged between 0.2-154 µg/kg was reported in a study conducted in Rwanda (Nyinawabali, 2013). Even though beans have low level of contamination, they are highly consumed in developing countries hence, can result into serious human health effects.

Lack of clear regulation, low awareness and weak monitoring mechanism of food safety along the food chain can influence the presence of high aflatoxins contamination along the value chain (Benkerroum, 2020; CRS, 2018; Kagot *et al*., 2019). The existence of aflatoxins in cereals and legumes in different areas of Africa, signifies the need for combined efforts among stakeholders in order to prevent contamination especially in food crops that are highly consumed.

2.8 Health Effects Caused by Aflatoxins Exposure

Aflatoxins are carcinogenic and cause other potential adverse health effects (Gong *et al.,* 2016). Aflatoxins B1 is the most potent and is responsible to acute and chronic toxicity (Magnussen & Parsi, 2013).The population of Sub-Saharan Africa countries are at higher risks of exposure to aflatoxins due to high consumption of the susceptible crops such as maize and legumes (Benkerroum, 2020; Massomo, 2020; IARC, 2015). Consumption of contaminated commodities in Africa is increased because the rejected produce that do not meet the international standards come in African food and feed chains, leading to increased exposure to the toxins (Okoth, 2016). Exposures, both chronic and acute have adverse effects to human and animal health. Lack of surveillance mechanisms in most of the African Countries, the magnitude of the problem, level of aflatoxins contamination and exposure is also underreported, underestimated and under costed (Okoth, 2016).

2.8.1 Aflatoxicosis

Aflatoxicosis is the poisoning that results from ingestion of aflatoxins (Benkerroum, 2020). The two forms of aflatoxicosis exist which are acute severe aflatoxicosis which leads into

illness like vomiting, abdominal pains, jaundice, direct hepatic failures and or death, while chronic aflatoxicosis results from the ingesting of low to moderate levels of aflatoxins which is associated with prolonged effects that are asymptomatic and difficult to recognize (Okoth, 2016). Chronic aflatoxicosis is more prevalent than acute aflatoxicosis (Gong *et al.*, 2016). The adverse health effects of chronic aflatoxicosis include hepatocellular carcinoma (HCC) (Gong *et al.*, 2012; Liu & Wu, IARC, 2015, 2010; Smith *et al.*, 2017), immune suppression (Jiang *et al.,* 2005; Wild *et al.*, 2003) growth retardation (Makori *et al.*, 2018; Shirima *et al.*, 2013; Watson *et al.,* 2018). In a study that was conducted in Ghana indicated that there was an association between aflatoxins and anemia in women of reproductive age (Engmann, *et al.*, 2008; Shuaib *et al.,* 2010).

The most severe acute aflatoxicosis epidemic in human was reported in Kenya in 2004 whereby 317 cases and 125 deaths were reported (Probst *et al.*, 2007). Same scenario was reported in Tanzania in 2016 whereby 68 cases and 20 deaths were reported (Kamala *et al.*, 2018). The major cause reported was the consumption of home grown maize which was highly contaminated by aflatoxins up to 4400 µg/kg and 51 100 µg/kg total aflatoxins in Kenya and Tanzania, respectively (Kamala *et al.*, 2018; Probst *et al.,* 2007).

2.8.2   Hepatocellular Carcinoma (Liver Cancer)

Aflatoxins is the most potent natural hepatocarcinogen (Liu *et al.,* 2012). Liver cancer is among the leading cause of cancer death worldwide, ranked the second in 2012 caused more than 745 000 deaths (Ferlay *et al.*, 2015). The primary liver cancer predominantly hepatocellular Carcinoma (HCC) constitute more than 85%. The risk factors for HCC include chronic infection with hepatitis B virus (HBV) or Hepatitis C Virus (HCV), Aflatoxin exposures (Gong *et al.,* 2016). Others include life style factors such as smoking excessive alcohol intake (Kimanya *et al.*, 2021). Globally, more than 4 billion people are exposed to dietary aflatoxins (Lizárraga-Paulín *et al.,*2016). The higher risk of aflatoxins exposure is in developing countries of Africa and Asia where aflatoxins susceptible food such as maize, rice, and groundnuts are highly consumed (Liu &Wu, 2010). In 2016, Tanzania had estimates of 1480 (2.95 per 100 000 persons) new cases of aflatoxins induced liver cancer (Kimanya *et al.,* 2021). Evidence shows that aflatoxins exposure has significant association with development of liver cancer (Wang &Tang, 2005).

Moreover, the synergistic effects between aflatoxins and chronic hepatitis B has been stipulated to increase the risks of liver cancer (Liu *et al.*, 2012). The distribution of HCC aflatoxins induced cases are higher in Africa (40%) and Asia (27%), respectively (Liu & Wu,

2010). Also, these regions experienced high prevalence of HBV. Moreover, the risk of aflatoxins induced HCC is high in rural population compared to urban due to the fact that urban people consume more diversified diet and food may be inspected for contaminants (Liu & Wu, 2010). Likewise, the prevalence of HBV is higher in rural than urban. Chu *et al*. (2018) found high serum AFB1-albumin adduct levels were significantly associated with an increased risk of HCC in Taiwan (Chu *et al.*, 2018). Liu and Wu (2010) reported that the risk of HCC in individuals with HBV is 30 times higher compared to non-HBV. A study in Kenya indicated that school children who had higher aflatoxins exposure was associated with chronic hepatomegaly (Gong *et al.,* 2012). A study on adolescent aflatoxins exposure in China and Taiwan predicted that exposure to Aflatoxin B1 (AFB1) will impose a potential risk for liver cancer in adulthood (Chen *et al.*, 2001; Peng *et al.*, 2007). On the other hand, aflatoxin B1 has been associated with the progression of liver and common tumors in rodents, and there has been found a possible link to increased esophageal cancer (Lizárraga-Paulín & Miranda-Castro, 2006).

2.8.3   Impaired Growth

Growth impairment in children is still a public health problem in sub Saharan Africa. Even though growth flattering in infants and young children (IYC) is caused by multiple factors, evidences have shown that aflatoxins exposure is associated with growth impairment in infants and young children (Shirima *et al.,* 2015; Tesfamariam *et al.,* 2019). Infants and young children are exposed to aflatoxins through placenta, breast milk, and complementary food (Hernandez-Vargas *et al.,* 2015). However, in most developing countries there is a risk of multiple exposures due to poor feeding practices (Smith *et al*., 2015). Most infants are introduced to cereal based contaminated complementary foods earlier than six months of age (Ahlberg *et al*., 2018; Atongbiik *et al.,* 2017; Chen *et al.*, 2018; Gheysens, 2015; Jonsyn-Ellis, 2012; Kumi *et al.*, 2014; Magoha *et al.*, 2016; Smith *et al*, 2017; Watson *et al.,* 2018). Moreover, the contaminated cereal based food are also main ingredients for nursing mothers' food that eventually will expose their children to aflatoxins through breast milk (Maleki *et al.,* 2015).

Studies in Gambia showed that high aflatoxins exposure during fetal development and infancy stage is associated with growth flattering (Hernandez-Vargas *et al*., 2015; Turner *et al.,* 2007). Turner *et al.* (2007) found a significant association between maternal exposure and growth flattering in infants in first years of life. Likewise, aflatoxins have been associated with poor pregnancy outcomes such as still birth, pregnancy loss, and fetal growth (Smith *et*

al., 2017). Moreover, Gong *et al.* (2004) found a significant association between aflatoxins exposure and growth flattering (stunting and underweight) among young children in Benin. Significant association was found between aflatoxins exposure and growth of infants aged 6-23 months in Dodoma Tanzania (Makori *et al.,* 2018).

2.9 Strategies to Reduce/Control Aflatoxins Contamination in Food

Aflatoxin is very stable toxin, once formed in food it is very difficult to be removed therefore strategies to control contamination both at pre and at post-harvest are important. Even though aflatoxins contamination starts in the field but higher levels of contamination occur in the storage due sub - optimal storage conditions (Massomo, 2020).

2.9.1 Strategies to Control Aflatoxins at Pre-Harvest Stage

At pre-harvest the strategies to reduce fungal contamination and subsequently aflatoxins production include awareness creation, good agricultural practices like timely planting, maintaining optimal plant densities; maintaining plant nutrition and avoiding drought stress, control other plant pathogens; weeds, insect pests, proper harvesting and biological control (Lizárraga-Paulín *et al*., 2013; WHO, 2018).

Awareness raising is very important in the effort to minimize contamination and subsequently exposures from contaminated foods (Magembe *et al.*, 2016). The awareness campaign will be more effective if the responsible agricultural researchers and health professionals will be knowledgeable about the toxins and work collaboratively (Jolly *et al*., 2009). Farmers and food handlers who are trained and aware can be important promotors to educate public about the harmful effects of the aflatoxins (Achaglinkame *et al*., 2017; Magembe *et al.*, 2016). Additionally, levels of awareness among farmers can also facilitate in identification and planning the aflatoxins mitigation measures (Ayo *et al*., 2018). Awareness on the aflatoxin, its health effects, control methods might help to reduce contamination as people might adhere to the recommended measures (Achaglinkame *et al.*, 2017).

Timely planting and application of inputs on time enable plants to have enough rainfall for health growth and resist pests and diseases (Atehnkeng *et al*., 2017). Also crop rotation between high susceptible with less susceptible to aflatoxigenic fungi is recommended to avoid inoculum in the field (Codex Alimentarius Commission [CAC], 2014).

Plant density and proper plant arrangement per unit are vital for nutrients and water but this depend on the type of crop and environmental condition (Achaglinkame *et al.*, 2017).

Excessive plant densities can stress plants, increasing their susceptibility to fungal infection (Leslie & Logrieco, 2014). The over dense of maize crop caused the increase in moisture content and nutritive stress hence the susceptibility to mycotoxins increases (Krnjaja *et al*., 2019; Marete *et al*., 2020).

Crops produced under stress are more prone to infestation by aflatoxins producing fungi (Marete *et al.*, 2020). There was a high aflatoxin contamination in 2009 in Kenya which indicated a correlation between drought and aflatoxins contamination (Mahuku *et al.,* 2019). Drought stress and aflatoxins were lowered in irrigated crops compared to un-irrigated (Abbas *et al.*, 2009). Moreover, maintaining proper plant nutrition and other key inputs reduce crops stress and hence reduce the fungal infestation (Atehnkeng *et al.*, 2017; Krnjaja *et al.,* 2019)

Excessive weeds can deplete soil moisture and compete on plant nutrients that make plant stressed and vulnerable to aflatoxin fungi (Leslie *et al.,* 2014). Likewise, insects infestation can create wounds on the crop that lead to invasion by fungi (Atehnkeng *et al*., 2017). Moreover, insects act as carrier of the aflatoxin fungi and or spores (Marete *et al*., 2020) .

The biological control method to reduce aflatoxin contamination involves the application of competitive non-toxigenic strains of *Asperigillus sp* to the soil of the developing crop (Chulze *et al.*, 2015; Lizárraga-Paulín *et al.*, 2013). The non- toxigenic fungi work by competing for nutrients, space, and the potential production of inhibitory metabolites with the aflatoxin producing fungi (Peles *et al.*, 2021; WHO, 2018). The use of non-toxigenic *A. flavus* have been found to reduce aflatoxins by more 80-99% in peanut and maize in the field and its effect has been carried over to the storage (Atehnkeng *et al.,* 2014; Chulze *et al*., 2015; Peles *et al.*, 2021). In Argentina the native atoxigenic showed a remarkable impact on the control of aflatoxin both in the field and storage of peanut (Chulze *et al.*, 2015).

2.9.2   Strategies to Control of Aflatoxin at the Post- Harvest Stage

Aflatoxins contamination reduction strategies at post-harvest has been widely reported including complex strategies such as chemicals i.e. nixtamalization, mechanical sorting with near-infrared spectroscopy to simpler methods that can be adopted easily by subsistence farmers (Kamala *et al.,* 2018). Strategies that use chemicals like calcium chloride, alkaline, hydroxides and bicarbonates can reduce aflatoxin content up to 84%-95% but some chemicals impair the nutrition quality of the products (Pandey *et al.,* 2019). Also high temperature cooking and steeping have effects on nutrition quality of the products (Lizárraga-

Paulín *et al.,* 2013). Strategies like timely harvesting, proper drying. sorting, dehulling, washing, adequate storage and control of pests (Lizárraga-Paulín *et al.*, 2013; Marete *et al.,* 2019; Shanakhat *et al.*, 2018). These strategies are simple, cost effective and easily adopted.

Timely harvesting immediately after the crop maturity is important to reduce aflatoxins contamination (Hell & Mutegi, 2011; Logrieco *et al.,* 2021). Delayed harvesting has been documented to expose grains to insects and rodents infestation hence increase fungal growth and aflatoxins production (Koskei *et al.,* 2020; Leslie *et al.*, 2014; Marete *et al*., 2019). Additionally, when maize are left to dry in the field they can be re-wetting by rain which may increase moisture content of the grains hence, facilitate fungal growth and subsequently mycotoxins production (Atukwase *et al.,* 2009; Kos *et al*., 2018; Udomkun, *et al.*, 2018). The delayed harvests reduced moisture but not to the required level for safe storage.

Grain moisture is among the important parameters to be considered during storage of grains (Chen *et al.*, 2018; Manandhar *et al.,* 2018; Ng'ang'a *et al*., 2016). Rapid and proper drying of cereals and legumes with moisture content about 10-13% is considered safe for long storage (Achaglinkame *et al.,* 2017; Logrieco *et al.,* 2021). At this level the growth of aflatoxins fungi and the formation of toxins in the storage grains is limited (Atongbiik *et al.*, 2017; Kamala *et al.*, 2018; Manu *et al*., 2019). Moreover, after the adequate drying, the temperature and moisture content of the stored grains should be monitored and maintained (Leslie *et al.*, 2014). Drying grains outside the field and off the ground reduce aflatoxins contamination (Kamala *et al*., 2018; Manandhar *et al.*, 2018). Adequately dried grains are rarely attacked by insects hence limit fungal growth (Kamala *et al.,* 2018; Nukenine, 2010). Kamala *et al.* (2016) reported that drying grains on elevated area reduces fungal contamination.

Aflatoxins contamination is heterogeneous distributed in certain portion of seeds hence separating the damaged or infected grains from the health contribute the reduction of levels of formed toxins and subsequently reduce the spread of aflatoxins producing fungi (Kamala *et al.,* 2018). In addition, sorting limit, the infected grains in the food chain hence reduce contamination. Hand sorting reduced aflatoxin level by 97% of AFB1 in contaminated white maize (Matumba *et al.,* 2015).

Dehulling involves the sprinkling water in maize to removal of the outer layer of the maize traditionally it was done using pestle and motor but it has been replaced by machine dehuller (Matumba *et al.*, 2015; Mutungi *et al.,* 2008). It is also a preceding procedure during maize milling. Aflatoxins are engrained in the outer layer of the maize, hence dehulling removing

most of the toxins in maize hence reduce contamination and subsequently dietary contamination (Kilonzo *et al.*, 2014). Dehulling reduced up to 92% of the aflatoxins in maize (Matumba *et al.*, 2015). In preparation of *Muthokoi* a tradition dehulled maize meal in Kenya it was reported lower aflatoxins levels in dehulled maize meal (*Muthokoi)* and maize meal compared to the intact maize kernels (Kilonzo *et al.,* 2014). Dehulling and whitening of rice reduced aflatoxins up to 97%, the high contamination was found in hull and bran (Castells *et al* ., 2007).

Most of the fungal contamination at the postharvest are due to inadequate storage (Drahansky *et al.*, 2016; Shabani *et al.*,2015). Fungal contamination is higher in tropics and sub tropics due to high temperature, humidity and poor aeration in the stores (Hell & Mutegi, 2011). It is documented that aflatoxins contamination in food increase with storage period (Suleiman *et al.,* 2017; Udomkun *et al.*, 2018). Aflatoxins levels in maize stored for more than six months were higher than their counter parts stored for two to six months (Kaaya & Kyamuhangire, 2006). Also, contamination of food may occur during transportation and processing (Gheysens, 2015; Leslie *et al.,* 2014; Lizárraga-Paulín *et al.*, 2013; Lutfullah & Hussain, 2012). Storage of grains in well ventilated store is recommended to reduce aflatoxins (Phokane *et al.,* 2019). Storage facilities play vital role to reduce or enhance fungal contamination (Drahansky *et al.*, 2016). Subsistence farmers and grain handlers in developing countries store grains in ordinary sacks or bags such as polypropylene which are not air tight (Kamala *et al.*, 2016; Sasamalo *et al.*, 2018; Shabani *et al.*, 2015). Polypropylene type of storage bags have been documented to facilitate fungal growth and aflatoxins production by increasing moisture contents and also allowing insects pests infestation (Mboya *et al.,* 2011; Shabani *et al.,* 2015).

On the other hand, hermetic storage facilities have been reported to reduce fungal proliferation and subsequently aflatoxins production (Danso *et al.*, 2018; PACA, 2012). Ng'ang'a *et al.* (2016) reported no changes in aflatoxins contamination was observed in the maize sample stored with moisture content below 13% for 35 weeks in Purdue Improved Crop Storage (PICS) bags. However, aflatoxins levels increased in samples stored in polypropylene and jute bags (Ng'ang'a *et al.*, 2016). In a study conducted in Meru and Makueni in Kenya found that the hermetic storage significantly reduced the increase in aflatoxin compared to polypropylene bags (Walker *et al.*, 2018). During high relative humidity grains stored in and jute bags gain moisture and increase chances of aflatoxigenic fungi growth.

Insects infestation increase humidity in the stored grains through their metabolic activities. Insects spread fungal spore through their movements (Manu *et al*., 2019). Additionally, damaged grains increase the chances of fungal contamination. Sasamalo *et al*. (2018) found high aflatoxins contaminations in food grains with high pest infestation. Farmers and grain sellers use various techniques to control insects and fungi infestation mainly is the application of synthetic pesticides. Pesticide can be effectively to reduce the risk of aflatoxins contamination (Leslie *et al*., 2014). Timely application of pesticides is recommended to prevent insect and fungal infestation (Leslie *et al*., 2014). Kamala *et al*. (2016) found that aflatoxin contamination was five times higher to maize farmers who did not apply pesticides compared to those who applied. Farmers who applied pesticides in their stored grains had fewer problems of aflatoxins contamination (Kamala *et al*., 2018).

However, the increased pest infestation particularly in food grains (cereals and legumes) contribute the indiscriminate use of pesticides in food grains both at pre and post-harvest hence food grains become a potent source of pesticide residues which lead to pesticide residues exposures (Akoto *et al*., 2013; Amvrazi, 2011).

2.10   Pesticide Residues Contamination in Food

Generally, there is a drastic shift in agricultural activities globally from traditional to non-traditional methods which have increased the reliance on pesticides to increase productivity (Hyseni, 2018). Pesticide is chemical substance or mixture of chemical substances intended for preventing, destroying, repelling or mitigating any pest (United States -Environmental Protection Agency [US-EPA], 2017). They can be either natural extract from plant or synthetic (Kim *et al.,* 2016). Some of the pesticide are harmful and can cause adverse effects to human and animal health (Kim *et al.,* 2016).

The estimated pesticide-related deaths worldwide are: 200 000 per year where 99% of these occur in developing countries despite the fact that the utilization of pesticide in these countries is only 25% (Hyseni, 2018; UNHRC, 2017)

The use of pesticides in agricultural production is a common practice in most of the developing countries due to existence of various pests and diseases causing pre and post-harvest losses (Kaushik *et al.,* 2009; Zanella *et al,* 2012). In Tanzania the use of pesticide is on the rise and the trend is expected to continue if alternative measures are not taken (URT, 2018). In 2006, 682 different pesticide were registered in Tanzania, which increased to 874 and 1114 in 2011 and 2018, respectively (URT, 2018). This trend must be considered in

terms of the possible impacts on peoples' life and food chains. Some common pesticides, which have been registered for use in Tanzania, include insecticides, fungicides, herbicides, acaricides, avicides, and rodenticides as shown in Table 1. Insecticides, fungicides, and herbicides account for about 90% of all pesticide usage in agricultural activities in Tanzania (URT, 2018).

Table 1:   Registered pesticide in Tanzania

Type of pesticides	Action/activity	n
Insecticides	Manage insect and arthropod	433
Fungicides	Destroy fungi	321
Herbicides	Destroy unwanted plant (weeds)	294
Acaricides	Inhibit the growth of mites and insects	56
Avicides	Manage birds	2
Rodenticides	Manage rodents	8

Lahr *et al*. (2016) and URT (2018)

Many smallholder farmers in Tanzania still use traditional storage methods despite large investments on improved storage technologies (Mutungi & Affognon, 2013). Nevertheless, the use synthetic pesticide has dominated other strategies to reduce losses. However, inappropriate treatment and use of adulterated pesticides affects its efficacy (Mutungi & Affognon, 2013). These might have contributed pesticide residues in the food grains hence, making it a potential source of pesticide residues through consumption of exposed diet (Adegbola *et al.*, 2012; Bajwa & Sandhu, 2014).

A survey conducted in Southern Agriculture Growth Corridor of Tanzania in 2016 along the higher maize production regions in the Southern part of Tanzania (Rukwa, Morogoro, and Iringa), indicated that cereals and legumes consume a lot of pesticides compared to other crops (Lahr *et al.,* 2016). Unacceptable level of various pesticide in raw and cooked maize flour was found in Dodoma and Ruvuma regions, including highly toxic and hazardous pesticide such as DDT metabolites (DDE) and endosulfan (Mahugija & Kayombo, 2017). Another study found pesticide residues in ready to eat food (rice, stiff porridge, beans and pigeon pea) in Dar-es-Salaam. Pesticide residues of g-HCH, pp-DDE, pp-DDT and Chlorpyrifos were detected in 29% of samples analysed, such as rice (50%), beef (42.1%), stiff porridge (8.6%), and beans (6.7%) (Ndengerio-Ndossi & Cram, 2005). In most of these samples, DDT and its metabolites were among the pesticide residues detected indicating that DDT is still used even though it has been banned for several year (Ndengerio-Ndossi &

Cram, 2005).

In the study which was conducted in Ghana residues of Organochlorine, Organophosphate and pyrethroids were found in maize and cowpea (Akoto *et al.*, 2013). The mean residual concentration of βHC, β endosulfan, p' p -DDE and pp DDD were above the EU MRL in both maize and cowpea. However, the health risks estimation shown that residues of heptachlor, dieldrin, endrin, β-endosulfna, c-chlordane and chlorfenvinphos found in maize were above the Acceptable Daily Intake (Akoto *et al.*, 2013). Similarly the levels of heptachlor and p, p-DDD found in cowpea also exceeded the Acceptable Daily Intake (Akoto *et al.*, 2013).

In another study conducted in Cameroon Organochlorine pesticides were detected more frequently and in higher concentrations, ranging from 0.02 to 0.01 mg/kg for endosulfan in millet to 9.53 to 4.00 mg/ kg lindane in maize. Organophosphorus compounds, was detected at concentrations varying from 0.04 to 0.03 mg/ kg for pirimiphos methyl to 0.23 to 0.38 mg/ kg for malathion in maize (Jean *et al.,* 2017). Permethrin was found only in maize at 0.39 to 0.23 mg/kg. In this study majority of samples contained multiple residues of both organocholrine and organophosphate. More than 75% of samples contained pesticide residues above the maximum residue limit (MRL); indicating a potential health risk related to consumption of these grains (Jean *et al.,* 2017).

2.11   Pesticide Residue Exposures

Pesticide residues are described as pesticide or metabolic products of pesticide that may remain after the application of pesticide (Dieterle *et al.*, 2004). Pesticide residues have become a major issue to farmers, government and consumers as they cannot be detected easily before or after consumption (Alphonce & Alfnes, 2012). The potential routes of pesticide exposure include the consumption of pesticide-contaminated foods and drinks, direct skin contact, and inhalation (Weng & Black, 2015). Unintentional exposure to pesticide is also common and dangerous especially in cases of unfamiliarity with pesticide or where farmers are unable to manage pesticide and dispose of them properly (Hajjar, 2012; Hyseni, 2018).

Dietary ingestion is a major route of exposure due to increased pesticide residues in a variety of food and drinks including; raw and cooked fruits, vegetables, water, cereals, and legumes (Kiwango *et al.*, 2018; Mahugija *et al*., 2017; Mahugija *et al*., 2017; Mtashobya *et al.*, 2017). Environmentally persistent pesticide residues basically enter the food chain through water,

air, and soil (Mtashobya, 2017; Ronchi & Danieli, 2008). Contaminated grains are often fed to animals leading to contaminated animal products like milk, meat, and eggs that constitute a substantial risk of exposure (Grewal *et al.,* 2017; Ronchi & Danieli, 2008). It is worrying that some of the detected pesticide residue concentration levels in food exceed the recommended MRL.

Exposure to pesticides can be chronic or acute where chronic exposure refers to a prolonged period of being exposed to a low dose of pesticide which can be more harmful and may result in chronic toxicity hence serious chronic health problems (Genon, 2013). The highly energetic young men and women who are involved in most farming activities like spraying pesticide, weeding, pruning, and harvesting are also likely to be at more risk from the prolonged durations of exposure to pesticide (Lahr *et al*., 2016).

Food consumption studies are scarce in developing countries hence pesticide dietary exposure data are also limited. However, studies on pesticides exposure due consumption of cereals and or legumes are scarce. The few exposure studies available mainly focusing on vegetables and fruits (Kariathi *et al*., 2017; Kiwango *et al.*, 2018; Mahugija *et al.*, 2017; Mtashobya, 2017) .

In Bekwara Nigeria in 2008 two children were reported died and 112 people hospitalized due to consumption of *moi-moi* (a Nigerian tradition food made of steamed beans) and beans *w*hich was preserved with harmful pesticide (Shaibu, 2008). The food samples revealed to contain high levels of organophosphate (Fenithrothion and chloropyrifos) and carbamates, that are highly toxic pesticide (Shaibu, 2008). Same scenario happened among 120 girls' students of Government girls secondary school in Gombe northern Nigeria who were hospitalized after consuming a beans meal suspected to have been preserved with poisonous chemicals which was later on confirmed was high levels of lindane an organchlorine pesticide commonly called gammallin. Gammallin was a toxic chemical which was illegally used to harvest fish (Shaibu, 2008). However, most farmers and other pesticide-handlers are not aware of the health risks associated with pesticide exposure and the lack of precautionary equipment has continued to be common practice in LMICs (Ngowi *et al.*, 2007; Nishant & Upadhyay, 2016).

2.12   Health Effects Due to Exposures to Pesticide Residues

The significant effects of pesticide on agricultural productivity and the reduction of post-harvest losses worldwide are well documented (The World Bank, 2015). On the other hand,

indiscriminate use of these chemicals potentiates negative effects on humans health (Fantke & Jolliet, 2015). These effects differ depending on the duration, levels, and routes of exposure as well as the pre-existing health status of individuals (Lynn, 2005). Additionally, physiological conditions and immaturity may increase the vulnerability of young children and adolescents to the deleterious health effects of exposure to pesticide (Hyseni, 2018).

Pesticide like organochlorine and pyrethroids are linked to endocrine disruption which interfere with nervous, immune and cardiovascular systems, and are likely to induce teratogenicity, carcinogenicity, and mutagenicity (Stoker & Kavlock, 2010). Likewise the organophosphates pesticide have inhibitory effects on the acetylcholinesterase enzyme (Kapeleka *et al.,* 2016). Acetylcholinesterase is an important enzyme in the nervous system that terminates nervous impulses by catalyzing the hydrolysis of neurotransmitters (Lionetto *et al.,* 2013). Farmers (15.6%) in Arusha and Iringa who were exposed to pesticides had acetylcholinesterase activity levels below the TPRI acceptable levels of 24.5 U/g Hb (Kapeleka *et al.,* 2019).

However, the long term exposure to pesticide has been linked to various chronic diseases and their co-morbidity such as obesity, diabetes mellitus (Everett & Matheson, 2010; Patel *et al.,* 2010; Son *et al.,* 2010; Mostafalou & Abdollahi, 2013; Daniels *et al*., 2018). Chronic respiratory diseases and asthma (Henneberger, 2014; Hernandez, 2011), Cardiovascular diseases (Chan *et al.,* 2007; Ha *et al.*, 2007; Ljunggren *et al.,* 2014), and cancer (Amr *et al.,* 2013; Flower *et al.*, 2004). Others are reproductive disorders, neurodevelopment disorders and Parkinson's, Alzheimer's (Owens & Feldman, 2010).

A study in Ethiopia showed that organophosphates were associated with respiratory illnesses among farm workers (Negatu *et al*., 2017). Another study in Costa Rica found a positive association between organophosphate pesticide and the respiratory health of indigenous women (Fieten *et al.*, 2009). Lerro *et al*. (2015) demonstrated a positive association between organophosphates pesticides and cancer among spouses of pesticide applicators in Iowa and California.

Mrema *et al.* (2017) identified the most prevalent diseases among women in the horticultural regions with high pesticide use in Tanzania included upper respiratory infections, hypertension, gynecological diseases, rheumatoid and joint diseases, pregnancy complications, skin infection, non-fungal bronchial asthma, and diabetes mellitus. The regions which have high pesticide consumption in Tanzania are Arusha, Kilimanjaro,

Manyara, Morogoro, Njombe, Iringa, Mbeya, Ruvuma, Dodoma, Kagera, Mwanza, Tanga, and Dar-es-salaam (Mrema *et al.,* 2017).

Acute exposure to pesticides has been associated with adverse health effects such as dizziness, muscular pain, wheezing, coughing, sneezing, itching, skin disease, breathing difficulties, nausea and eye diseases (Lekei *et al.*, 2016). Nearly one-third (32.4%) of workers in tea company in Tanzania, self-reported symptoms of pesticide exposure like headache, skin irritation, chest pain and coughing (Kapeleka *et al.,* 2016). Similar symptoms have been reported for small scale farmers in Uganda who felt sick after the regular application of pesticides (Oesterlund *et al.*, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1   Study Area Description

This study was conducted in Kilimanjaro region, which is located in the North Eastern part of Tanzania. It lies in the South of the Equator between latitudes 2°25 and 4°15 and longitudes 36° 2́30" and 38°10́45 East of the Greenwich. Kilimanjaro region covers an area of 13 209 km^2 which is 1.4% of the area of the entire country. Administratively, the region is divided into six districts namely; Rombo, Mwanga, Same, Hai, Moshi and Siha (Fig. 2). The total population of the region in 2012 was 1.6 million; of these adolescents'/youth population aged 10-19 years covers 24.6% which is 403 896 youth (NBS, 2016). Majority of the population (75%) in the region depend on agriculture and livestock keeping for livelihood (National Bureau of Statistics, 2016). Kilimanjaro region was purposively selected due to greater varieties of food produce such as bananas, maize, beans, rice, potatoes and sorghum that might have influenced dietary diversification (NBS, 2016; Maghimbi, 2007). Also, the region has the highest number of boarding high schools in Tanzania with sixty boarding schools both private and public (Table 2). Of all schools, 54 schools were located in rural and semi-rural while six schools were in urban area. However, the study considered 31 schools (government and private) in rural areas of Kilimanjaro region. Of the selected, districts; Moshi, Siha, Rombo and Hai are relatively fertile with average rainfall of more than 1000 mm per year that support own crop production (URT, 2017). The remaining two districts, Same and Mwanga are relatively dry and own crop production is limited resulting to somewhat food deficit (International Federation of Red Cross Crescent Societies [IFRCCS] 2013; URT, 2013).
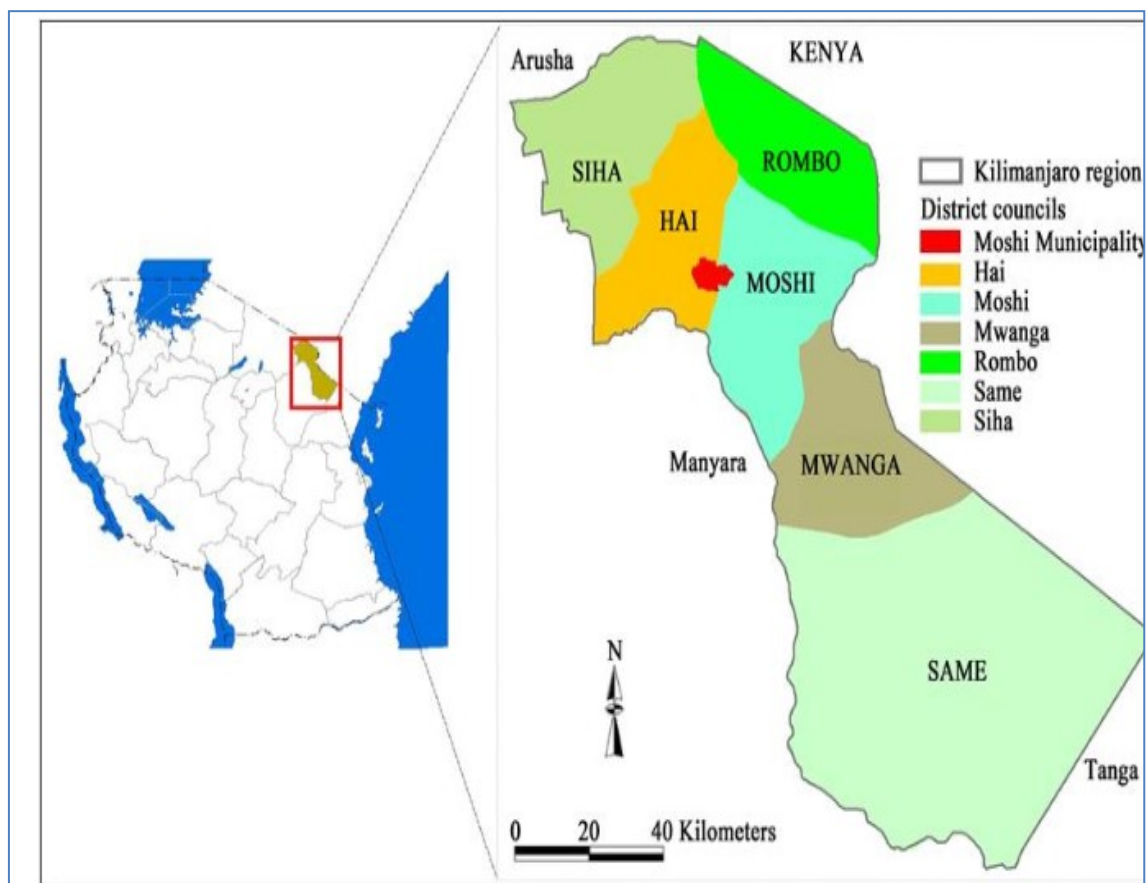
Figure 2:   **Map of the study area Kilimanjaro region**

3.2   Sampling Procedure

Multistage random sampling technique was used whereby boarding high schools in each district were clustered into private or government (Gali *et al.,* 2017). List of boarding high schools in each district was obtained from the respective district office. In each district at least two and at most three schools were randomly selected from each cluster using excel random numbers hence 15 public and 16 private schools were recruited to make a total of 31 schools (Fig. 3).

Table 2:   **Boarding high schools in rural areas of Kilimanjaro region**

District	Public	Private	Total
Rombo	2	3	5
Hai	3	6	9
Siha	4	3	7
Moshi (R)	6	11	17
Same	2	5	7
Mwanga	5	4	9
Total	**22**	**32**	**54**

Six schools were sub-sampled for assessments of food consumption and nutrition status and knowledge and awareness on diet related diseases, aflatoxins, and pesticide exposures. These

schools were randomly selected from the two clusters of private and public schools and hence three schools from each cluster were involved, making a total of six schools.

3.3 Sample Size Determination

Sample size was calculated using Fischer's formula (Pourhoseingholi *et al*., 2013) considering the prevalence of overweight (12.7%) among adolescents in secondary schools (Chinenere, 2014).

$$N = \frac{(Z)^2 * P(1-P)}{D^2}$$

Whereby

N= Desired sample size of Secondary school adolescents

Z= Standard deviation usually 1.96 which corresponds to 95% confidence level.

P= Proportion of secondary school adolescents who are estimated to be overweight (12- 19 years old)

D = Absolute precision required

$$N = \frac{(1.96)^2 \{0.127(1- 0.127)\}}{(0.05)^2} = 170.29$$

Based on non-response rate of 10% was considered and the calculated sample size was 170/0.9= 189 adolescents. The random selection of participants was done using balloting technique (Ogum *et al.,* 2018). One hundred and sixty-four adolescents aged 16-19 years agreed to participate in the study.
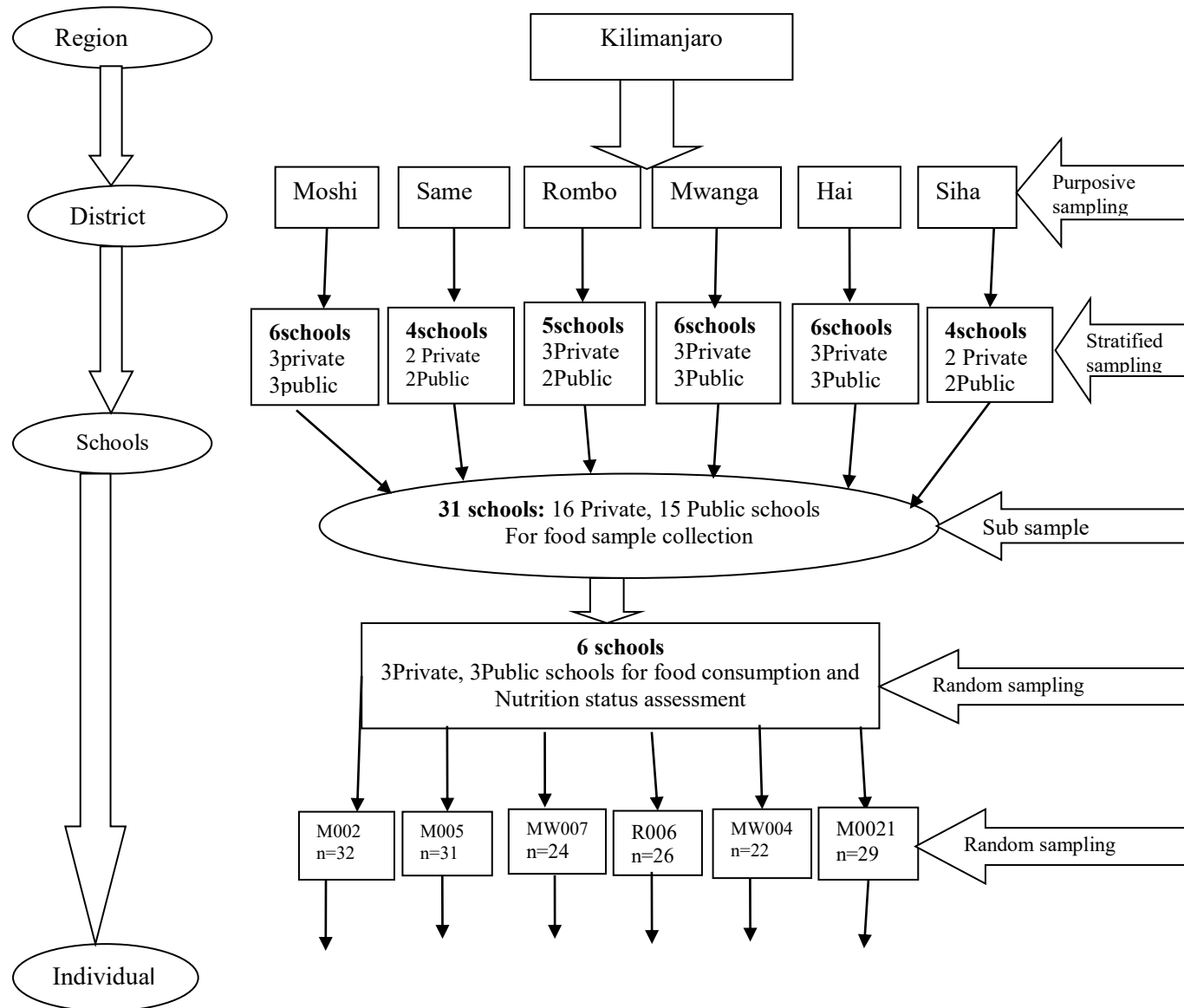
Figure 3: **Schematic presentation of sampling procedures**

3.4 Inclusion and Exclusion Criteria

Study participants included boarding high school students aged 16-19 years, who consented to participate in the study. Adolescents who did not consent were excluded from the study. Teachers/staff responsible for school meals who consented were included.

3.5 Data Collection

In-depth interview and survey guided with structured and semi-structured questionnaires were used to collect participants' demographic information, food handling, school feeding, knowledge and awareness on diet related disease, aflatoxins knowledge and awareness. The questionnaire consisted of both open and closed-ended questions. The questionnaires were designed in English language, translated to Swahili to make it easier for the participants to understand. The questionnaires were pre-tested among ten students in one boarding school, which was not part of this study. Necessary modifications were made before administering the same to the study participants.

3.6 Assessment of Nutrition Status

3.6.1 Anthropometry

Anthropometric measurements of weight, height and age were taken by the researcher and trained research assistant. Weight was taken with bare feet and light clothing and participant standing upright in self-calibrating digital weighing scale (CAMRY). Height measurements were taken by using stadiometer (ADEL MS-120 Medical Scale) measured to the nearest 0.1 cm with the participants standing upright with bare feet. All measurements were taken in a designated room to minimize interference. Anthropometric measurements were used to calculate BMI and BMI for age which was computed by WHO Anthro plus software (version v1.0.4). Body Mass Index (Weight kg/height (m^2) is a reflection of the height and weight of an individual. It is a simple method to determine nutrition status based on weight, height, age and sex (Adesina *et al.,* 2012). WHO (2007) criteria was used to classify nutrition status. The BMI for Age z-scores categories were used for adolescents aged below 19 such as <-2 SD (Underweight), ≥-2SD and ≤+1SD(normal), >+1SD and ≤ +2SD (Overweight), >+2SD (Obese) while BMI was used to classify nutrition status of adolescents aged above 19 years such as <18.5 (Underweight), 18.5 to 24.9 (Normal), 25.0 to 29.9 (Overweight) ≥ 30 (Obese), (De Onis *et al*., 2007). Stunting or short stature was determined by Height for Age Z score less than -2 standard deviation (De Onis *et al.,* 2007).

3.6.2 Haemoglobin Levels

Anemia is one of the most serious and common nutritional deficiency disorders of public health concern in developing countries (Parbey *et al.*, 2019). It is the condition of low levels of hemoglobin in the blood (Lynch, 2011). Iron is a main component of haemoglobin and over half of all anemia globally are estimated to be caused by iron deficiency (Lynch, 2011). Hemoglobin level was determined on site by using portable battery operated HemoCue Hb 201[+] system (HemoCue AB, Angelholm, Sweden). A drop of capillary finger prick blood was drawn and filled in a HemoCue - Cuvette and results read within 10 minutes. Haemocue was tested before commencement of every session. A trained laboratory technician did hemoglobin levels determination. The WHO (2011) hemoglobin cut off points for adolescents was used to assess anemia as indicated in Table 3.

Table 3:   Haemoglobin levels to define adolescents anaemia

Category	Hemoglobin cut off points in g/dl	
	Girls	Boys
Non-anemia	12 or above	13or above
Mild anemia	11.0 -11.9	11.0 to 12 .9
Moderate anemia	8 - 10.9	8 - 10.9
Severe anemia	Below 8	Below 8

WHO (2011)

3.7   Dietary Intake

Dietary data were collected using Food Frequency questionnaire (FFQ) and 24 hours' dietary recall methods. Food Frequency questionnaire was used to assess the feeding frequency and habitual diet of adolescents. The method was chosen because it is simple and inexpensive to apply in low resource settings (Food and Agriculure Organisation [FAO], 2018). A modified FFQ comprised a list of 50 food items confined in twelve food groups, namely: (a) cereals and cereal products, (b) roots, tubers and plantains, (c) legumes, nuts and seeds, (d) meat and meat products (e) eggs, (f) milk and milk products, (g) oils and fats, (h) fruits, (i) vegetables, (j) beverages, (k) snacks and (l) others was used (FAO, 2010). Each participant was asked to recall how often a certain food and drink was consumed per day, weekly; monthly, rare and never /not eaten. The questionnaire was adopted from Zack *et al.* (2018) with modification to suit the study population. Twenty-four hours' dietary recall was used to collect dietary information and amount of food and beverages consumed over the past 24 hours inside and outside schools. Participants were asked to recall all food and drinks taken from when they woke up in the morning up to the time before they went to sleep at night. School meals were

prepared using local recipes, information on the cooking methods and ingredients used were obtained from teachers/staff responsible for school meals and or cooks. Portion size was estimated using cooked food for common food items like stiff maize porridge and the estimated portion size was determined using kitchen digital scales. Other common households' utensils were calibrated and measures translated into grams equivalent. The household measures included; cups, plates, measuring jars, spoons, customary packing size and solid food in pieces or slices; were used to estimate portion size. Seasonal fruits were purchased to estimate fruits portion size. Nutrients intake were calculated by the Nutri-survey for windows software version 2007 (Erhardt, 2007).

3.8   Knowledge and Awareness on Diet Related Diseases

Data on awareness and knowledge were collected using questionnaires. Participants were asked whether they were aware of diet related diseases. Information collected on knowledge included views of participants on types and causes of diet related diseases. It is important to understand adolescents 'knowledge on these diseases because dietary related diseases are influenced by lifestyle behaviors including dietary that begin at adolescence stage. However, getting adolescent knowledge on diet related diseases will provide evidence to recommend for intervention.

Responses were recorded and knowledge among adolescents and staff responsible for school meals was assessed based on the correct responses. Multiple responses analysis was performed to analyze participants' responses on the types and causes of diet related diseases. The total response per each diet related diseases mentioned and the causes were weighted in 100% each as multiple responses. The responses were grouped into quantiles. The interpretation of knowledge was done based on the scores obtained that is those who scored below <25.5% were considered having poor knowledge; 25.5–<50.5% were having fair knowledge, (≥50.5%) were considered as having good knowledge (Melesse & Van den Berg, 2021).

3.9   Aflatoxins Contamination in Food and the Risk of Exposure among Adolescents

3.9.1   Food Samples Collection and Preparation

Samples of at least 3 to 4 of commonly consumed food such as maize flour, dehulled maize, rice and beans were collected from each of the 30 boarding schools from six districts of Kilimanjaro region namely Same, Mwanga, Moshi, Siha, Rombo and Hai. Due to

heterogeneity nature of aflatoxins and that most of the samples were in 100 kg bags, Multiple samples of cereals and legumes were collected each using respective probe (Nyangi *et al*., 2016). Samples were collected from different bags from the school store and were collected at different points such as top, middle and bottom of a bag (Nyangi *et al,* 2016) aggregate samples were thoroughly mixed and a sub sample of 1kg were obtained (Nyangi *et al*, 2016) (Fig. 4). A total of 119 food samples were collected and were grouped as samples of maize flour (=30), dehulled maize *(Kande)* (n= 30), rice (n= 30), and beans (n=29). Samples were collected into a dry tighten kaki envelops, labeled and transported to NM-AIST laboratory for analysis of aflatoxins. Samples were stored at 4°C prior to analysis of aflatoxins. Samples of dehulled maize, rice and beans were milled and homogenized using electrical grinding machine. Sub- sample of each milled grains was taken for aflatoxin analysis.

Figure 4: **Collection of food samples from 100 kg sacs at the store of one of the studied boarding schools**

3.9.2 Validation of the Method Performance

Prior to analysis of samples, calibration of the machine was done by analysing aflatoxins standards (Aflatoxin analytical mix of B1 G1 B2, G2 standards) at a concentration of 20, 15 10 and 5 µg/kg. The analytical method was validated for accuracy and precision (National Association of Testing Authorities ([NATA], 2012).

Three samples of maize, beans and rice were spiked with aflatoxins standards (G1, G2, B2 and B1) at three levels concentration of 0.5, 1.0 and 2.0 µg/kg to determine recovery percentage. Spiked samples were extracted and analyzed under the same condition and procedures as the samples.

Recovery percentage was calculated by using the following formula:

% Recovery = (Detected concentration/ spiked concentration) x100   (N ATA, 2012)

The detection and quantification limits were determined using visual method as described by Sengul (2015). The lowest detectable concentration and Limit of quantification were determined by spiking samples with a gradual decrease of the concentration of aflatoxins standards (G2, G1, B2, and B1).

3.9.3 Determination of Aflatoxins Contamination of Food Samples

Determination of aflatoxins in food samples was done by the method reported by Stroka *et al.* (2000) with slight modification. Briefly, 500 g of food grain samples were milled and homogenized using electrical grinding machine. Twenty-five grams of each milled and homogenized sample was weighed into in a blender jar and mixed with 100 mL of mixture of methanol: water (60: 40 v/v) as extraction solution. The mixture was blended vigorously for 30 minutes, filtered using Whatman no.1 filter paper (Sigma-Aldrich). Then 4 mL of the filtrate was diluted with 8 mL Phosphate Buffer Saline (Fig. 5). The diluted extract was loaded into a syringe connected to the immunoaffinity column and allowed to pass through the column to nearly dryness. The syringe and immunoafinity column were rinsed using 10 mL distilled water (HPLC grade). Then, elution of bounded aflatoxins was done using 1mL of acetonitrile into a glass amber vial.

Derivatization of aflatoxins was done by mixing 400 µL of eluent with 600 µL derivatizing reagents (70:20:10 v/v/v of H_2O: TFA: Acetic Acid). The mixture was placed in a water bath at 65°C for 15 minutes, then cooled at room temperature. After cooling, 10 µL was injected into High Performance Liquid Chromatography (HPLC) (Shimadzu, Tokyo, Japan) fixed with florescence detector set at wavelength of 450 nm emission and 365 nm excitation. The HPLC column (C18 Spherisorb 80-3 ODS-1, 5 µm, 4.6 × 150 mm) was set at a temperature of 40°C. The mobile phase was composed of Water: Methanol: Acetonitrile (60:30:10 v/v/v) at a flow rate of 1 mL/minute. The running time was 15 minutes.

Figure 5:   **One of the laboratory procedure during the extraction of aflatoxins from food samples**

3.9.4   Assessment of Dietary Aflatoxins Exposure

Dietary aflatoxins exposure among adolescent was calculated using aflatoxins contamination, amount of food consumed and body weight (Kimanya *et al.,* 2014; Shephard, 2008) as follows:

$$\text{Dietary exposure (ng/kg bw/day)} = \frac{\text{Food consumption (g/day)} \times \text{contamination (ng/g)}}{\text{Body weight of the subject (kg)}}$$

For the case of AFB1 all undetected samples were assigned contamination level of half the limit of detection (EFSA, 2011). Amount of cereal and legumes consumed was estimated by using recipes stipulated in the Tanzania food composition table whereby 100 g of cooked thin porridge was estimated to contain 10 g of raw maize flour, and 100 g of stiff porridge contain 32.1 g of raw maize flour (Lukmanji *et al.*, 2008; Makori *et al*., 2018). Likewise, the

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estimated amount of raw rice and beans consumed as part of 100 g of cooked rice and beans was 5 g and 60 g, respectively (Lukmanji *et al.*, 2008).

3.9.5   Risks of Aflatoxins Exposure

Margin of exposure (MOE) was used to characterize risks of aflatoxins exposure in individual adolescent (EFSA, 2007; Benford *et al.*, 2010) by dividing the Benchmark Dose Lower Limit (BMDL) for aflatoxins B1 by the exposure of individual adolescent. Adolescents with MOE less than 10 000 indicated that the risk of exposure to aflatoxins is of public health concern. This is also translated as aflatoxins exposure above 0.017 ng/kg/bw/day (obtained by dividing 170 ng/kg/bw/day by 10 000) (Benford *et al.*, 2010; EFSA, 2012). The lower risk is observed when MOE is above 10 000 or if aflatoxins exposure is below 0.017 ng/kg /bw/day (EFSA, 2012).

3.10   The Risk of Pesticide Exposure among Adolescents

3.10.1 Food Sample Collection and Preparation

Due to resource limitations, analysis of pesticide residues was done in 60 samples which were samples of maize flour (n=30) and beans (n=30). These samples were selected due to their high frequency of consumption as part of school meals. The sample were collected from 30 schools in six districts of Kilimanjaro region. At least 500 g of samples of maize flour and beans each were collected using probe sampling technique then wrapped into aluminum foil and packed into labeled khaki envelopes and were transported to Government Chemist Laboratory Authority (GCLA) where the analysis of pesticides residues was performed. The samples of beans were ground and homogenized using electrical grinder. A sub- sample of each sample was taken for pesticide residue analysis.

3.10.2 Method Performance and Quality Assurance

The method performance was validated according to European guidelines (SANTE, 2018). The analysis was performed to determine the quality parameters such as recovery, limit of detection and quantification, and linearity. A calibration curve was prepared from mixed standards of Cypermethrin, Permethrin, Pirimiphos methyl, Deltamethrin, Gamma-cyhalothrin and Chloropyrifos each at a concentration of 1.00, 0.5, 0.25, 0.125, 0.0625, 0.03125 mg/kg. Recovery was determined by spiking the blank samples of beans and maize with mixed standards (Cypermethrin, Permethrin, Pirimiphos methyl, Deltamethrin, Gamma-

cyhalothrin and Chloropyrifos) each at concentrations of 0.05, 0.1, 0.2 mg/kg. The spiked samples were extracted and analyzed in the same conditions and procedures as the test samples.

% Recovery = (Recovered concentration /spiked concentration) x100

The LOD was determined by spiking the samples by gradual lowering the concentration of the standard of pesticide The limit of detection (LOD) was determined as the lowest concentration of the analyte from the sample which can be detected but not necessarily quantified (Shrivastava, & Gupta, 2011; Bernal, 2014). The LOQ was determined as the lowest concentration that could be quantified at acceptable accuracy and linearity (Shrivastava, & Gupta., 2011). The LOD and LOQ were determined as 3:1 and 10:1 signal to noise ratio, respectively (Gupta, 2015).

3.10.3 Determination of Pesticides Residues in Maize and Beans Samples

The pesticides residues extraction was done following Swedish Ethyl Acetate method (SweEt) (Andersen & Poulsen, 2013) with some modifications. Briefly, 200 g of beans samples were ground and homogenized using electrical grinder (IKA A11 Basic S2). Then, 5 g of ground sample of beans and maize flour each weighted into 50 mL polypropylene centrifuge tube. Then 10 mL of distilled water was added followed by 10 mL of Ethly Acetate with 1% Acetic Acid (1:10v/v). The mixture was vortexed for 30 seconds. Then 10 g of Sodium Sulphate was added and vortexed for 10 seconds. The mixture was extracted in ultra sound bath (5510 Branson) for 30 minutes, then the mixture was centrifuged (Hettich Universal 320) for 5 minutes at 4000 rpm (Fig. 6)

Solid Phase Extraction (SPE) was performed by conditioning the SPE cartridges valves by adding enough volume of a mixture of Methanol: Acetonitrile (1:1v/v) in a 500 mL conical flask. The mixture was sonicated for 15 to 30 minutes. The valves were left to dry and C18 columns were attached and eluted with 6 mL of Methanol 2 to 3 mL at a time followed by 6 mL distilled water 2 to 3 mL at a time. Then 1 mL of the supernatant was allowed to pass through the column until no more solvent. Then, the elution of the pesticides residues was done by using 3 mL of Dichloromethane 1 mL at a time via collecting test tubes. The eluent was concentrated by Nitrogen evaporator to 1 mL then transferred into 1.5 mL vials for Gas Chromatography –Mass Spectrometer (GC-MS) for analysis of pesticides residues

Pesticide residues were analyzed by GC-MS (Agilent 7890B equipped with 7697 auto-sampler coupled to 7000D triple quadruple MS system. The pesticide residues were separated on VF-5ms (ultra- inert) 30 x 0.25 mmx 0.25 µm column and detected by Q-TOF triple quadrupole Mass spectrometer (MS/MS) operating with electron energy at 70ev, Source temperature at 250°C and transfer line at 280°C, inlet temperature 280°C. The injection volume 1 µL. All pesticides residues were detected and quantified in the Multiple Reaction Monitoring (MRM).

Figure 6:   Some of the procedures performed during pesticide residues extraction

3.11   Data Analysis

Data collected were entered cleaned, coded and analyzed in IBM Statistical Packages for Social Sciences (SPSS version 23). The descriptive statistics such as frequency, mean and median were computed. Association of continuous data was performed by using Independent t –test. Chi square for independence was performed to determine the comparison between categorical variables. Univariate and Multivariate logistic regression was performed to determine the association between independent and dependent variables.

3.11.1 Demographic Data

Descriptive statistics such as frequency mean and standard deviation was calculated using SPSS and presented in tables.

3.11.2 Anthropometry Data

Data for Anthropometry measurements that were collected were further computed by WHO Anthro plus 2007 BMI and Z scores were calculated. The percentage were obtained from SPSS to determine prevalence of underweight, overweight, obesity and stunting. Means were calculated for weight, height and BMI. Chi-Square test was used to test comparison of

nutrition status between boys and girls, private and public schools and adolescents' nutrition status in different.

3.11.3 Dietary Intake Data

Nutri survey for windows 2007 version was used to compute daily energy and nutrients intake (Erhardt, 2007). Mean of nutrients intake was determined. Independent t-test was used to test differences in nutrients intake among adolescents. Significant differences were set at P-value (<0.05).

3.11.4 Knowledge and Awareness on Diet Related Diseases

Multiple responses analysis was used to determine knowledge of diet related diseases and knowledge and awareness among participants. Frequencies and percentage were obtained to determine good, fair and poor knowledge among participants.

3.11.5 Data on Aflatoxins and Pesticide Residues Contamination

Descriptive statistics such as frequency, percentage and median were obtained to determine occurrence of aflatoxin and pesticides residues in food crops used by schools.

3.12 Ethical Consideration and Logistics

Ethical clearance was sought from the National Health Research Ethics Sub-Committee (NatHREC) of the National Institute for Medical Research (NIMR), Tanzania with reference number NIMR/HQ/R. 8a/Vol. IX/2730. Permission to conduct this study was also sought from other relevant authorities such as the Regional Administrative Secretary, District Executive Directors and respective schools' authorities. The purpose of the study was well explained to the school administrators, students and matrons or teacher responsible for meals before commencement. Assent was sought from students and caregivers' signed the consent form. The caregivers consent for the students below 18 years while students aged 18 years and above signed consent form once they agreed to participate in the study. Participating schools and subjects were given special code to maintain anonymity and confidentiality of the information obtained was assured and participation was voluntary.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Demographic Characteristics of the Participants

In total, 164 students from high schools participated in this study. Out of which 112 (68.3%) were female and 52 (31.7%) male. Male adolescents were reluctant to participate as compared to girls that makes them fewer compared to their counterparts. The age of respondents at recruitment ranged between 16 and 19 years with mean (±SD) of 18.3 (± 0.7). About 144 (87.8%) of the study participants were in the age group of 18 to 19 years and 20 (12.2%) were in the age of 16 to 17 years. Participants mothers; 69 (42.1%) had secondary level education while 47 (28.6%) and 48 (29.3%) had primary and tertiary levels of education respectively. Forty-eight percent were involved in business as means of livelihood while 48 (29.3%) and 37 (22.6%) were employed and farmers, respectively. Participants' fathers; 75 (45.7%) had secondary education while 38 (23.2%) and 51 (31%) had primary and tertiary levels education, respectively. Likewise, participants' fathers, 65 (39.6%) were formally employed with the rest involved in business 62 (37.8%) and farming 37 (22.6%). Based on the mode of school ownership, 87 (53.0%) respondents were in private and 77 (47.0%) respondents were in public schools (Table 4).

Table 4:   Social- demographic characteristics of participants (n=164)

Characteristics	n	%
Mean(Standard deviation) age (years)		
Age (years)		
16-17	20	12.2
18-19	144	87.8
Gender		
Female	112	68.3
Male	52	31.7
Education level of the mothers		
Primary education	47	28.6
Secondary education	69	42.1
Tertiary education	48	29.3
Education level of the fathers		
Primary education	38	23.2
Secondary education	75	45.7
Tertiary education	51	31
Occupation of the mother		
Employed	48	29.3
Farmer	37	22.6
Business	79	48.1
Occupation of the father		
Employed	65	39.6
Farmer	37	22.6
Businessman	62	37.8
District		
Rombo	26	15.9
Moshi	94	57.3
Mwanga	44	26.8
School ownership		
Private	87	53.0
Public	77	47.0
School type		
Girls only	65	39.6
Boys only	20	12.2
Co-education	79	48.2

4.1.2   Dietary Practices, Knowledge and Awareness on Diet Related Diseases

(i)   Meals Planning in Boarding High Schools in Kilimanjaro Region

Responses from staff responsible for school meals indicated that planning of school meals 17 (54.4%) involved head teacher, teacher responsible for meals and students. Some respondents claimed that their school menu has been put in place for a long time and has never changed except for minor modification done in special circumstances. Regarding guidelines on school

meals planning. Majority, 25 (80.6%) respondents said there was no guideline for planning school meals while 6 (19.4%) they do not know if the guidelines exist or not (Table 5). Moreover, the school meal planning is highly dependent on the funds provided by the government to the schools and school cash flow for government and private schools, respectively.

Table 5: **Meals planning in boarding secondary schools in Kilimanjaro region**

Variable	Attribute	n	%
Planning of school meal	Head teacher, teacher responsible for meals and students	17	54.4
	School management team	4	12.9
	School committee	10	32.3
Guideline for school meals	No	25	80.6
	I don't know	6	19.4
Basis for planning of school meals	Availability	13	41.9
	Income	6	19.4
	Nutrition	5	16.1
	Availability and Income	7	22.6

(ii)   School Meals Schedule and Amount of Food Consumed

Table 6 presents the number of meals and amount of school meals consumed by adolescents per day. Majority 157 (95.7%) of the adolescents reported to have fixed daily meal schedules, 103 (62.8%) reported to consume two to three meals per day. Moreover, the median and range of amount of maize flour, dehulled maize, rice and beans consumed per day was 51 – 638 g, 173.1 - 454 g, 15.7-42.2 g and 121.2-595.2 g per day, respectively.

Table 6: **School meals schedule and amount of food consumed**

Variable	n	%
Number of meals per day		
2-3 meals	103	62.8
4 meals	61	37.2
Fixed daily meals schedule		
Yes	157	95.7
No	7	4.3
Amount of food consumed	**Median**	**Range**
Maize flour	151.4 g	51.04- 638.15 g
Dehulled maize	103.8 g	173.1- 454 g
Rice	21.1 g	15.65- 42.20 g
Beans	214.0 g	121.20-595.20 g

(iii)   Foods Consumed by Adolescents in Boarding Secondary Schools

Table 7 presents types and frequency of food consumed by adolescents in boarding schools. Starchy staples particularly cereals and cereal products were frequently consumed at least once per day between five to seven days per week; predominantly maize stiff porridge was ranked the first by 99 (60.4%) of the respondents followed by maize thin porridge consumed by 94 (57.3%) of the respondents. Other cereal-based food like boiled rice was consumed by 106 (64.6%) of respondents in the frequency of 3 to 4 days per week. Roots tubers and plantains were rarely consumed by 13 (7.9%). Fleshy foods were occasionally consumed, slightly more than half of the respondents 84 (51.2%) consumed fleshy food once per day for 1 to 2 days per week. Eggs and dairy were rarely consumed by 11 (6.7%) and 4.9% respondents, respectively. Almost all respondents 158 (96.3%) consumed kidney beans twice per day in seven days. Nuts and seeds were rarely consumed by 20 (12.2%) respondents. Cooking oils (Sunflower, OKI, Korie, and Safi) were used in preparation of all school meals and hence were consumed by all respondents. However, fruits were consumed 1 to 2 days per week by 78 (46%) of the respondents. ripe bananas were highly consumed 59 (36%) respondents followed by oranges consumed by 51 (31.1%) respondents. Leafy vegetables were consumed by 57 (34.7%) respondents for 1 to 2 days per week. Beverages especially black tea (tea without milk) were consumed daily by 71 (43.3%) respondents at least once per day as part of breakfast or mid-morning snack. Processed snacks like biscuits, crisps and sweets were rarely consumed by 36 (22.0%) of the respondents. Other items like chili sauce, tomato sauce and honey were consumed by fewer respondents 22 (13.4%) for 1 to 2 days per week.

Table 7: **Food consumed by adolescents in boarding secondary schools**

Food group	Meals consumed per day		Frequency of consumption						
	1meal/ day	2meals/ day	1-2 days/wk	3-4 days/wk	5-6 days/wk	7days /wk	1-2 days /month	Rare	Never
	n (%)	n (%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Starchy staples									
Maize thin porridge	99 (60.4)	24(14.6)	13 (7.9)	16 (9.8)	36 (22.0)	58 (35.4)	-	-	41 (25)
Maize stiff porridge	132 (80.5)	26(15.9)	8 (4.9)	51(31.1)	57 (34.8)	42 (25.6)	-	-	6 (3.7)
Dehulled maize and beans	160 (97.6)	4(2.4	73(44.5)	66 (40.2)	7(4.3)	14 (8.5)	-	-	4 (2.4)
Rice	164(100)	-	34 (20.7)	106 (64.6)	24 (14.6)	-	-	-	-
Bread /burns/chapati	135(82.3)	-	17 (10.4)	27 (16.5)	38 (33.2)	53 (32.3)	-	5 (3.0)	24 (14.6)
Roots, tubers, and plantains	14(8.5)		10 (6.1)	4 (2.4)	-	-	3(1.8)	13(7.9)	134 (81.7%)
Fleshy food 1	120(72.2)	-	84 (51.2)	5(3.04)	-	-	36 (22)	27(16.4)	12 (7.31)
Eggs	14(8.5)		11 (6.7)				3 (1.8)	30(18.3)	120 (73.2)
Dairy	13 (7.9)	-	5 (3.0)	8 (4.9)	-	-	-	-	151(92)
Legumes	-	164(100)	-	3 (1.8)	3 (1.8)	158(96.3)		-	-
Nuts and seeds	29(17.6)	-	15 (8.1)	10 (6.1)	5 (7.9)	4 (2.4)	2 (1.2)	20 (12.2)	108 (65.8)
Fats and Oils		164(100)	-	-	-	164 (100)	-	-	-
Fruits2	96(58.5)	-	78 (46)	28 (17.0)	4 (2,4)	11(6.7)	-	35 (21.3)	8(4.8)
Leaf Vegetables	71(43.3)	-	57 (34.7)	8 (4.2)	-	6 (3.7)	-	48 (29.3)	45 (27.4)
Beverages	150(91.5)		8 (4.9)	30 (18.2)	41 (25)	71(43.3	-	-	14 (8.5)

Food group	Meals consumed per day		Frequency of consumption							
	1meal/ day	2meals/ day	1-2 days/wk	3-4 days/wk	5-6 days/wk	7days /wk	1-2 days /month	Rare	Never
	n (%)	n (%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Processed snacks	62(37.8)	-	30 (18.3)	21(12.8)	3 (1.8)	8 (4.9)	-	36 (22.0)	66 (40.2)
Other items	43(26.2)	-	5 (3.0)	9 (5.5)	7 (4.3)	22 (13.4)	-	9 (5.5)	119 (72.6)

(-) represents a particular food item was not consumed.

n is the number of adolescents consumed a certain type of food

[1]includes meat, poultry and fish

Fruits [2] include ripe bananas, oranges and avocados

(iv) Energy and Nutrients Intake

Data from the 24 hours' dietary recall indicated that the average energy intake was 1311 Kcal for male and 1473 Kcal for female, which was less than the Recommended Daily Allowances (Table 8). The mean intake of 80.7 g protein and 471.9 g carbohydrates was slightly above the Recommended Daily Allowance for the adolescents. An independent samples t-test was performed to compare the average nutrients intake between male and female. There was a significant difference in the average intake of proteins, carbohydrates and fat between female and male adolescents ($P<0.001$). Male adolescents had significant ($P<0.001$) intake of proteins and carbohydrates 93.9 g and 535.6 g, respectively as compared to their female' counterparts. On the other hand, female adolescents had a significant intake of fat 89.0 g compared to male ($P< 0.001$).

The average intake of micronutrients 24.8 mg vitamin C, 9.2 mg iron, 134.5 mg calcium, and 4.3 mg zinc was below the RDA. Most adolescents did not meet the Recommended Daily Allowance for these micronutrients (Table 8). Seventy-five percent of adolescents were below the RDA for iron intake while 70.7% adolescents did not meet RDA for vitamin C. Likewise 164 (100%) and 159 (97%) adolescents did not meet RDA for calcium and zinc, respectively. There was a significant difference ($P=0.018$) in mean iron intake between male and female (Table 8). In terms of gender majority of female adolescents were below the RDA for micronutrients such as 98 (87.5%), 79 (71.0%), 112 (100%) and 109 (97.3%) for iron, vitamin C, calcium and zinc, respectively (Table 9). Independent-samples t-test was used to compare the mean intake among adolescents in age categories from 16 to 17 and 18 to 19 years. Consumption of various nutrients was not significant in the two age categories as indicated in Table 8.

Table 8:   Energy and nutrients intake of food consumed by adolescents in boarding secondary schools

Nutrients	RDA	Male M(SD) n=52	Female M(SD) n=112	t-test	P value
Energy (Kcal)	2162.70	1311.7(442.4)	1473.8(567.7)	-1.989	0.049
Protein (g)	52 &46	93.9 (28.4)	67.6(20.9)	5.986	<0.001*
Fat (g)	73.40	58.3 (32.3)	89.0(38.3)	-5.013	<0.001*
Carbohydrates (g)	308.70	535.6 (177.2)	408.3(117.6)	4.719	<0.001*
Iron (mg)	11 &15	10.6 (7.7)	7.8(4.9)	2.430	0.018*
Vitamin C (mg)	75.00	26.7 (53.8)	22.9(23.9)	0.485	0.630
Calcium (mg)	1300	139.2 (97.3)	130.8(81.6)	0.576	0.565
Zinc (mg)	11&9	4.6 (2.9)	4.0(2.0)	1.324	0.190

Nutrients	RDA	Age distribution		t-test	P value
		16-17 M(SD)n=20	18-19 M(SD) n=144		
Energy (Kcal)	2162.70	1482.2(544.9)	1414.1(535.2)	0.533	0.595
Protein (g)	52&46	69.4(19.4)	76.8(27.2)	-1.185	0.238
Fat (g)	73.40	95.2(39.6)	77.1(38.7)	1.955	0.052
Carbohydrates (g)	308.70	415.2(123.2)	453.3(154.1)	-1.058	0.292
Iron(mg)	11 &15	6.6(4.5)	8.9(6.2)	-1.642	0.103
Vitamin C (mg)	75	18.8(21.7)	24.8(37.5)	-0.705	0.482
Calcium (mg)	1300	135.6(117.2)	133.1(82.0)	0.119	0.906
Zinc (mg)	11&9	3.6(1.9)	4.3(2.4)	-1.327	0.186

Institute of Medicine (2011)

*Statistically significant (P<0.001), Nutrient intake computed by Nutri survey for windows 2007, Tanzania Food Composition table 2008, RDA represent Recommended Daily Allowances, ≥RDA above OR equal to the RDA, < RDA represents below RDA

Table 9: **Nutrients intake against school ownership and gender among adolescents in boarding high schools**

Nutrients	School ownership		P value	Gender		P value
	Private	**Government**		**Male**	**Female**	
Energy						
<RDA	78(89.7)	65(84.4)	1.004(0.316)	50(96.2)	93(83.0)	5.473(0.019)
≥RDA	9(10.3)	12(15.6)		2(3.8)	19(17)	
Protein						
<RDA	33(37.9)	28(36.4)	0.043(0.836)	8(15.4)	53(47.3)	15.505(0.001)
≥RDA	54(62.1)	49(63.6)		44(84.6)	59(52.7)	
Fat						
<RDA	36(41.4)	43(55.8)	3.423(0.064)	33(63.5)	46(41.1)	7.131(0.008)
≥RDA	51(58.6)	34(44.2)		19(36.5)	66(58.9)	
Carbohydrates						
<RDA	14(16.1)	18(23.4)	1.380(0.240)	7(13.5)	25(22.3)	1.775(0.183)
≥RDA	73(83.9)	59(76.6)		45(86.5)	87(77.7)	
Iron						
<RDA	72(82.8)	51(66.2)	5.949(0.015)	25(48.1)	98(87.5)	29.436(0.001)
≥RDA	15(17.2)	26(33.8)		27(51.9)	14(13)	
Vitamin C						
<RDA	64(55.2)	52(67.5.)	0.718(0.397)	37(71.1)	79(71.0)	0.007(0.935)
≥RDA	23(47.9)	25(32.4)		15(28.8)	33(29.5)	
Calcium						
<RDA	87(100.0)	77(100.0)	-	52(100)	112(100)	-
≥RDA	-	-		-	-	
Zinc						
<RDA	85(97.7.)	74(96.1)	0.353(0.553)	50(96.2)	109(97.3))	0.164(0.686)
≥RDA	2(2.3)	3(3.9.)		2(3.8.0)	3(2.7)	

Significance difference tested at 95 CI, P<0.005, Independent t- test, Nutrient intake computed by Nutri survey for windows 2007, Tanzania food composition Table 2008, RDA represent Recommended Daily Allowances, >RDA above OR equal to the RDA, < RDA represents below RDA

(v) **Knowledge and Awareness on Diet Related Diseases**

Staff responsible for school meals 20 (64.5%) were aware of the diet related diseases. Ninety percent 18 (90%) were able to mention types of diet related diseases such as kwashiokor, obesity, diabetes, hypertension and cancer. Kwashiokor had higher responses 10 (55.6%) responses indicating that staff responsible for school meals had a good knowledge on kwashiorkor while diabetes and obesity had 8 (44.4%) and 6 (33.3%) responses, respectively indicating fair knowledge. Meanwhile, cancer and hypertension got responses less than 25.5% each indicating staff had poor knowledge on these diseases. Furthermore, 18 (90%) were able to respond on the causes of diet related diseases. Responses were higher in overeating 7 (38.9%) responses and 6 (33.3%) responses on bad cooking oils which indicate staff had fair knowledge (Table 10).

Table 10: **Knowledge and awareness on diet related diseases among staff responsible for school meals**

Variable	n	%
Awareness on diet related diseases n=31		
Yes	20	64.5
No	11	35.5
Know types of diet related diseases (n= 18)*	**Frequency**	**%**
Obesity	6	33.3
Hypertension	2	11.1
Diabetes	8	44.4
Cancer	2	11.1
Kwashiokor	10	55.6
Know causes of diet related diseases (n=18)*	**Frequency**	**%**
Over eating	7	38.9
Bad fat/oil	6	33.3
Toxins	5	27.8
Inadequate nutrients intake	2	11.1

* Multiple responses

In this study, majority 127 (77.4%) of students were aware of diet related diseases. However, 125 (98.4%) students were able to respond on the types of diet related diseases such as obesity, hypertension, diabetes cancer kwashiorkor, marasmus, anaemia. The fair responses were in obesity, kwashiorkor, diabetes and marasmus indicating students had fair knowledge on these diseases. Moreover, 118 (92.9%) students were able to respond on the causes of diet related diseases. Mostly mentioned causes were 63 (53.4%) responses on inadequate nutrients intake and 35 (29.7%) responses on overeating indicating students had good and fair knowledge on inadequate nutrients intake and overeating, respectively (Table 11).

Table 11:   **knowledge and awareness on Diet related diseases among adolescents**

Variable	n	%
Awareness on diet related diseases n=164		
Yes	127	77.4
No	37	22.6
Know types of diet related diseases n =125	**Frequency ***	**%**
Obesity	61	48.8
Diabetes	33	26.4
Cancer	6	4.8
Hypertension	15	12.0
Kwashiokor	54	43.2
Anaemia	23	18.4
Marasmus	41	32.8
Others (Allergy, Rickets, Scurvy, and Goiter)	28	24.2
Know causes of diet related diseases n=118	**Frequency ***	**%**
Overeating	35	29.7
Lack of physical exercise	7	5.9
Too much fat	12	10.2
Too much carbohydrates	11	9.3
Lack of food	18	15.3
Too much sugar	13	11.0
Monotonous diet	3	2.5
Inadequate nutrient intake	63	53.4
Chemicals	1	.8

* Multiple responses

4.1.3   Nutrition Status among Adolescents in Boarding Secondary Schools

(i)   Nutrition Status

Mean height among adolescents was 160.9 cm. There was a significant difference in height between female and male adolescents (P<0.001). Male adolescents were taller 165.5 cm compared to female 158 cm. Mean BMI of female adolescents was significantly higher 23.8 ± (3.8) (P=0.022) compared to male 22.5± (3.1). Findings on adolescents BMI indicated that 41 (64.5%) were normal, while 2 (3.2%) underweight, 18 (29.0%) and 2 (3.2) were overweight and obese, respectively. Moreover, the nutrition status by BMI for Age z –scores showed that 23 (22.5%) were overweight, 8 (7.8%) obese and majority 71 (69.6%) were normal. Height for Age Z- scores indicated that 96 (94.1%) were normal and 6 (5.9%) Stunted (<-2SD) (Fig. 7).
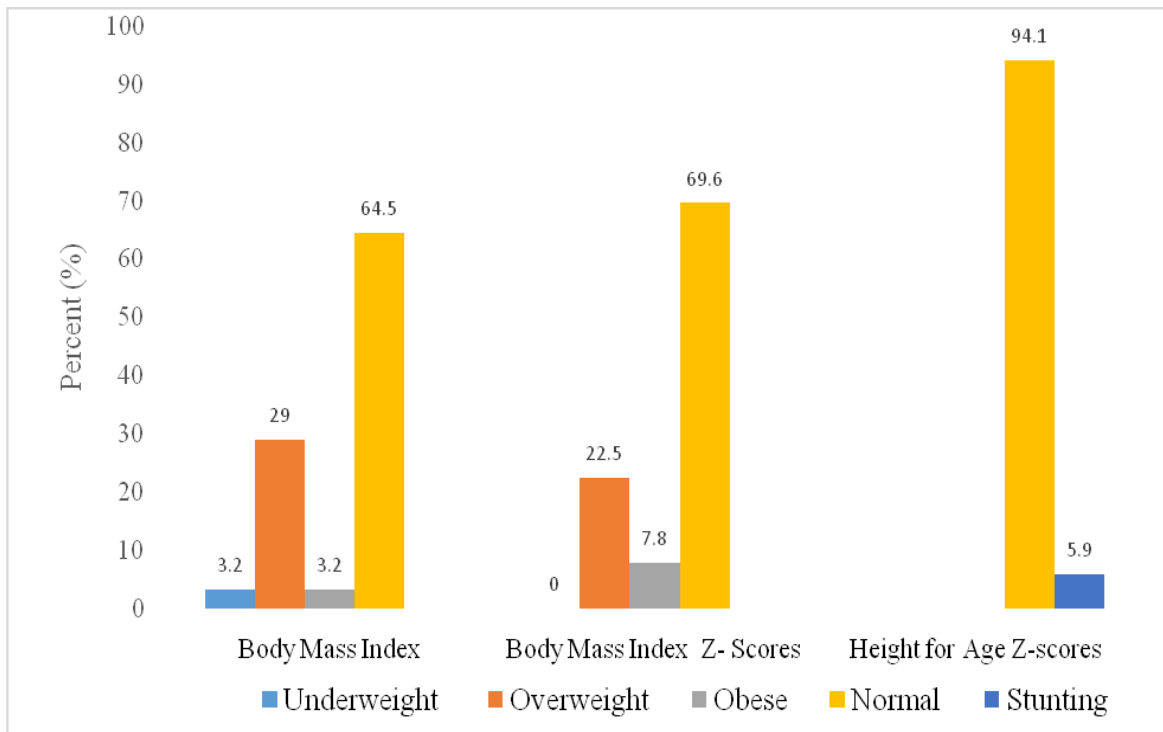
Figure 7: **Nutrition status of adolescents in Kilimanjaro region**

(ii)   Hemoglobin Levels

Anemia was observed among studied adolescents. The prevalence of anemia was 38 (23.2%) of the study participants. Mean hemoglobin level was 13.6 g/dl. There was a significant difference in mean hemoglobin levels between female and male adolescents (P<0.05). Table 12 gives details of anemia situation.

Table 12:   Distribution of anemia by gender among adolescents

Variable	Female n (%)	Male n (%)
Moderate anemia	14 (12.5)	1(2.0)
Mild anemia	17(15.2)	6(11.5)
Non anemic	81(72.3)	45(86.5)

Hemoglobin classification based on (WHO, 2011)

(iii)   Association between Socio-Demographic Factors and Nutrition Status among Adolescents

Results showed there was an association between socio demographic factors and BMI Z-Score. Occupation of the father had significant association with Body Mass Index Z- scores (P<0.05). Adolescents of employed parents had high proportion of overweight and obesity 7 (14.9%) and 8 (17%) compared to compared to adolescents of businessmen and adolescents with farmers' parents (p=0.017; χ^2=12.015) (Table 13).

Regarding the location, students from schools located in Moshi District had high proportion of overweight (14 =42.4%) compared to those from Rombo (3 =27.3%) and Mwanga District (1=5.6%). It was observed that private schools had more overweight adolescents (10=30.3%) compared to government schools (8=27.6%), the association was not statistically significant (Table 13).

Table 13: **Association between socio demographic factors and BMI among adolescen**ts

Characteristics	Body Mass Index (kg/m²) (n=62)					Body Mass Index Z Scores (n=102)			
	Under weight	Normal	Over weight	Obese	χ^2 (ρ-value)	Normal	Over weight	Obese	χ^2 (ρ-value
Gender									
Female	1 (2.6)	22 (56.4)	15(38.5)	1(2.6)	4.576(0.206)	48(65.8)	19 (26)	6 (8.2)	1.972(0.373)
Male	1 (4.3)	18 (78.3)	3 (13)	1(4.3)		23(79.3)	4 (13.8)	2 (6.9)	
Education of the mother									
Primary education	-	16 (69.6)	6 (26.1)	1(4.3)		15(62.5)	8 (33.3)	1 (4.2)	
Secondary education	-	17 (70.8)	6 (25)	1(4.2)	8.711(0.191)	34(75.6)	7 (15.6)	4 (8.9)	3.242(0.518)
Tertiary education	2 (13.3)	7 (46.7)	6 (40)	-		22(66.7)	8 (24.2)	3 (9.1)	
Occupation of the father									
Employed	-	8 (44.4)	9 (50)	1(5.6)		32(68.1)	7 (14.9)	8 (17)	
Farmer	1 (6.2)	12 (75)	3 (18.8)	-	7.344(0.190)	14(66.7)	7 (33.3)	-	12.015(0.017)
Businessman	1 (3.6)	20 (71.4)	6 (21.4)	1(3.6)		25(73.5)	9 (26.5)		
Occupation of the mother									
Employed	-	5 (41.7)	7 (58.3)	-		26(72.2)	6 (16.7)	4(11.1)	
Farmer	1 (6.2)	10(62.5)	5 (31.2)	-	8.999(0.174)	14(66.7)	7 (33.3)	-	3.902 (0.419)
Business woman	1 (2.9)	5 (73.5)	6 (17.6)	2(5.9)		31(68.9)	10 (22.2)	4 (8.9)	
District									
Rombo	1 (9.1)	7 (63.6)	3 (27.3)	-		11(73.3)	4 (26.7)	-	
Moshi	-	18 (54.5)	14 (42.4)	1 (3)	10.167(0.118)	39(63.9)	15 (24.6)	7 (11.5)	4.327 (0.364)
Mwanga	1 (5.6)	15 (83.3)	1 (5.6)	1(5.6)		21(80.8)	4 (15.4)	1 (3.8)	
Ownership									
Private	1 (3)	20 (60.6)	10(30.3)	2(6.1)	1.972(0.578)	35(64.8)	13(24.1)	6 (11.1)	2.060 (0.357
Government	1 (3.4)	20 (69)	8 (27.6)	-		36 (75)	10 (20.8)	2 (4.2)	

Statistical difference tested at 95 CI P<0.05, χ^2 test

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(iv)   Socio- demographic Factors Associated with Overweight /Obesity among the Boarding School Adolescents

Table 14 indicates univariate and multivariate logistic regression analysis to determine factors associated with adolescents overweight /obesity. Univariate logistics regression indicated that female adolescents had twice the risk of being overweight/obesity compared to male adolescents (cOR=2.4; 95% CI; 1.10, 5.34). (P=0.028). Adolescents of employed fathers had two times high risk of being overweight /obesity compared to adolescents of fathers who were farmers and business men cOR= 1.69 (95% CI; 0.69, 4.07**)**. Private schools' adolescents had double risk of being overweight /obese compared to their counter parts public schools cOR =1.58 (95% CI; 0.81, 3.09).   Moreover, adolescents from girls'only schools have significant four times higher risk of being overweight/obesity compared to their counter parts boys only and co- education cOR= 4.29 (95% CI: 1.14, 16.08).

After adjusting for all independent variables in multivariate regression analysis indicated that age between 18-19years aOR = 1.77(95% CI 0.55, 5.61), female sex aOR =1.99 (95%CI ;0.60, 6.61), employed father aOR =1.56(95% CI: 0.49, 4.96), and private school aOR= 1.77(95%CI 0.73, 4.27) were associated with obesity /overweight (Table 14).

Table 14: **Univariate and multivariate logistic regression to determine the association between overweight/obesity and Socio-demographic characteristics of the adolescents (n=164)**

Variable	N	n (%) Overweight/ obese	cOR (95%CI)	ρ-value	aOR (95%CI)	ρ-value
Age (years)						
16-17	20	6(30.0)	1		1	
18-19	144	45(31.2)	1.06 (0.38-2.94)	0.910	**1.77(0.55-5.61)**	0.336
Gender						
Male	52	10(19.2)	1		1	
Female	112	41(36.6)	**2.43 (1.10-5.34)**	**0.028**	1.99(0.60-6.61)	0.260
Education level of the mothers						
Primary education	47	16(34)	1		1	
Secondary education	69	11(15.9)	0.68 (0.31-1.53)	0.357	0.60(0.237-1.54)	0.289
Tertiary education	48	11(22.9)	1.06 (0.46-2.47)	0.888	0.92(0.29-2.99)	0.900
Education level of the fathers						
Primary education	38	12(31.5)	1	1		
Secondary education	75	24 (32.0)	1.02 (044-2.36)	0.964	0.91(0.37-2.26)	0.841
Tertiary education	51	15(29.4)	0.90(0.36-2.25)	0.826	0.89(0.33-2.44)	0.825
Occupation of the mother						
Farmer	37	12(32.4)	1		1	
Employed	48	17(35.4)	1.14(0.46-2.83)	0.774	0.72(0.18-2.83)	0.640
Business	59	22(37.3)	0.80(0.35-1.87)	0.613	0.64(0.21-1.94)	0.426
Occupation of the father						
Farmer	37	10(27)	1		1	
Employed	65	25(38.5)	**1.69(0.69-4.07)**	0.244	**1.56(0.49-4.96)**	0.451
Businessman	62	16(25.8)	0.94(0.37-2.36)	0.894	0.71(0.21-24)	0.578
School ownership						
Public	77	20(25.9)	1		1	
Private	87	31(35.6)	**1.58(0.81-3.09)**	0.184	**1.77(0.73-4.27)**	0.207

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Variable	N	n (%) Overweight/ obese	cOR (95%CI)	ρ-value	aOR (95%CI)	ρ-value
School type						
Boys only	20	3(15)	1		1	
Girls only	65	28(43.1)	**4.29 (1.14-16.08)**	**0.031**	**2.69(0.36-20.05)**	0.333
Co-education	79	20(25.1)	**1.92 (0.51-7.25)**	0.331	1.25 (0.20-7.72)	0.809
Meals Frequency						
2 -3 times a day	103	32(31.1)	1		1	
4times	61	19(31.1)	1.00(0.51-1.99)	0.992	0.77(0.33-1.77)	0.536

cOR: Crude odd ratio, aOR; Adjusted odd ratio, CI: Confidence interval, ρ-value <0.05)

(v)   Association between Socio-Demographic Factors and Hemoglobin Levels among Adolescents

Hemoglobin levels for girls were associated with demographic factors. Education levels of mothers' were significant associated with girls' hemoglobin P<0.05. Adolescents of mothers with primary education had high proportion (32.1%) of mild anemia compared to 14.7% tertiary education and (6.0%) secondary level (χ^2 =14.280, *p*=0.006). In regards to the location, there was a significant association (P<0.05). High proportion (4=44.4%) of moderate anaemia was observed for girls from boarding schools located in Mwanga District compared to (7=9.1%) in Moshi and (3=11.5%) in Rombo Districts (χ^2 =12.838, *p*=0.012). Likewise, girls in private schools had high proportion (14=25.5%) of mild anaemia compared to girls in government schools with proportion of (3=5.3%) (χ^2 =14.115, *p*=0.001) (Table 15).

Table 15:   **Association between socio - demographic factor and girls' hemoglobin levels (n=112)**

Characteristics	Total	Hemoglobin levels for girls students			χ^2 (ρ-value)
		Moderate anaemia	Mild anaemia	Non-anaemic	
Age (Years)					
16-17	19	2 (10.5)	2 (10.5)	15 (78.9)	0.536 (0.765)
18-19	93	12(12.9)	15 (16.1)	66 (71)	
Education of the mother					
Primary education	28	3 (10.7)	9 (32.1)	16 (57.1)	14.280 (0.006)
Secondary education	50	10 (20)	3 (6)	37 (74)	
Tertiary education	34	1 (2.9)	5 (14.7)	28 (82.4)	
Occupation of the father					
Employed	42	6 (14.3)	6 (14.3)	30 (71.4)	2.325 (0.676)
Farmer	21	4 (19)	4 (19)	13 (61.9)	
Businessman	49	4 (8.2)	7 (14.3)	38 (77.6)	
Occupation of the mother					
Employed	34	4 (11.8)	3 (8.8)	27 (79.4)	3.441 (0.487)
Farmer	19	3 (15.8)	5 (26.3)	11 (57.9)	
Business woman	59	7 (11.9)	9 (15.3)	43 (72.9)	
District					
Rombo	26	3 (11.5)	1 (3.8)	22 (84.6)	12.838 (0.012)
Moshi	77	7 (9.1)	15 (19.5)	55 (71.4)	
Mwanga	9	4 (44.4)	1 (11.1)	4 (44.4)	
Ownership					
Private	55	10 (18.2)	14 (25.5)	31 (56.4)	14.115 (0.001)
Government	57	4 (7)	3 (5.3)	50 (87.7)	

Statistical difference tested at 95 CI, P <0.05) χ^2 test

4.2.1 Aflatoxins Contamination and Risks of Aflatoxin Exposure among Adolescents

(i) Types and Sources of Grains used in Boarding Schools

Findings from staff responsible for school meals (n=31) indicate that, majority 28 (90.3%) use the following grains with percentage obtained through purchase; maize 24 (77.4%), rice 31 (100%) and beans 31 (100%), while only 7 (22.6%) of maize is obtained from own production. Purchase of school grains was done monthly 19 (61.3%). Schools with own grains production, 4 (57.1%) reported to have harvested and stored maize for six months or more prior to data collection (Table 15). Fifty-eight percent of schools sourced food from Kilimanjaro region with occasional purchase 10 (32.3%) from outside the region and mostly from Tanga region 6 (60%) and the rest 4 (40%) shared between Arusha, Mbeya, Kahama and Singida regions while, 3 (9.7%) failed to establish sources of the grains purchased. Moreover, schools with storage facilities, make abundant purchase during harvesting season and store for time-to-time use (Table 16).

Table 16: Types and sources of grains used for school meals

Variable	Attribute	n	%
Type of grains used for school meals	Maize, rice and beans	28	90.3
	Maize, rice, beans and wheat	3	9.7
Main source of maize	Purchase	24	77.4
	Own production	7	22.6
Main source of rice	Purchase	31	100
Main source of beans	Purchase	31	100
Storage time	3 months ago	3	42.8
	6 to 9 months ago	4	57.1
Purchasing period	1month	19	61.3
	3months	6	19.4
	6 months and above	6	19.4
Grains purchased outside Kilimanjaro	Yes	10	32.3
	No	18	58.1
	Don't know	3	9.7
Source of purchased grains	Tanga region	6	60.0
	Other regions (Arusha, Mbeya, Kahama and Singida	4	40.0

(ii) Food Handling in boarding Schools

Table 17 indicates that, more than half of the boarding high schools 16 (51.6%) store grains in ordinary polypropylene sacks or bags, 15 (48.4%) use both silos and ordinary polypropylene sacks whereby silos were mainly used for long term storage of maize.

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Majority 25 (80.6%) of the staff reported no use of pesticides, while 6 (19.4%) applied pesticides once per year and mainly insecticides 4 (66.7%) while 2 (33.3%) applied both insecticides and rodenticides. Moreover, 16 (51.6%) believed to have purchased grains which were not treated with pesticides while 14 (45.2%) did not know whether the purchased grains were treated or not (Table 17).

Table 17:   Food grains handling practices at schools

Variables	n	%
Storage of grains		
Ordinary polypropylene sacks/bags	16	51.6
Silos and polypropylene sacks/bags	15	48.4
Control of pests		
Application of pesticides	6	19.4
Do not apply pesticides	25	80.6
Type of pesticides used		
Insecticides (Atellic, shumba, shamba,)	4	66.7
Insecticides and rodenticides (actellic /simba, phostoxin)	2	33.3
Withdraw period before using treated grains		
1 to 3 month	4	66.6
4 to 6 months	2	33.4
Purchase treated grains		
Yes	1	3.2
No	16	51.6
Don't know	14	45.2

(iii)   Preparation of Grains for School Meals

Staff respondents 13 (41.9%) from schools that prepare its own maize flour used for stiff and thin porridge reported to winnow and sort the grains before milling, while 11 (35.5%) purchased commercially processed maize flour. Winnowing and washing were reported in the preparation of rice 27 (87.1%), beans 22 (71.0%) and de-hulled maize 17 (54.8%) used for *Kande* (Table 18).

Table 18:   Preparation of grains before cooking (n=31)

Variable	n	%
Maize flour		
Commercially processed maize flour	11	35.5
Winnowing and sorting	13	41.9
Winnowing and decortications	7	22.5
Dehulled maize		
Winnowing and Sorting	3	9.7
Winnowing and washing	17	54.8
Commercially dehulled maize	11	35.5
Beans		
Winnowing and washing	22	71.0
Winnowing, sorting and washing	9	29.0
Rice		
Winnowing and washing	27	87.1
Winnowing, sorting and washing	4	12.9

(iv)   Method Performance and Quality Assurance

Calibration curve of r^2=0.99 was obtained. The mean percentage recoveries for total aflatoxins were 101, 110 and 86 in rice, maize and beans respectively. The LOD and LOQ for aflatoxins G2, G1, B2 and B1 were determined as indicated in the Table 19.

Table 19:   Retention time, Limit of detection (LOD) and Limit of Quantification (LOQ)

Aflatoxin type	Retention time (minutes)	LOD (µg/kg)	LOQ (µg/kg)
G2	5.88	0.28	0.35
G1	7.65	0.39	0.42
B2	9.76	0.27	0.32
B1	13.77	0.25	0.27

(v)   Occurrence of Aflatoxins in Food used for School Meals

Aflatoxins were detected in 91 (76.5%) of 119 food samples used for school meals. Of the detected samples, 96.7% ,93.3%, 60% and 55.2% samples of dehulled maize, maize flour, rice and beans, respectively were contaminated. The overall median and range for total aflatoxins contamination was 2.30 µg/kg and 0.20 to 438.53 µg/kg, respectively. The highest concentration range of 0.59-438.53 µg/kg was found in maize sample while the lowest range of 0.20-3.41µg/kg was found in rice. Aflatoxin B1 was detected in 41 (34.5%) of the samples with a contamination range between 0.44 to 35.88 µg/kg. The highest AFB1 contamination of 35.88 µg/kg was found in dehulled maize while the lowest contamination of 0.44 µg/kg was found in rice (Table 20).

Likewise, the aflatoxins contamination levels was compared to the maximum limits of 5 µg/kg for AFB1 and 10 µg/kg for total aflatoxins set by Tanzania Bureau of Standards (TBS, 2014). Out of samples detected with aflatoxins, 11 (12.1%) exceeded maximum limit for AFB1, of which 6 (75%) were beans. Moreover, total aflatoxins in 12 (13.2%) samples exceeded maximum limit, of which 9 (32.1%) were samples of maize flour (Table 20).

Table 20: **Prevalence of aflatoxins contamination of food crops used in school feeding and its comparison to Tanzania regulatory limits**

Food type		Total aflatoxins				Aflatoxin B1			
	Number of samples	Positive sample n(%)	Median	Range	Contamination > 10 µ/kg n (%)	Positive sample n (%)	Median	Range	Contamination >5 µ/kg n (%)
Overall Median			2.3	(0.20, 438.53)			1.14	(0.44, 35.88)	
Rice	30	18(60.0)	2.01	(0.20, 3.41)	-	2(11.1)	0.47	(0.44,0.50)	--
Maize Flour	30	28(93.3)	3.59	(0.59, 438.53)	9(32.1)	20(71.4)	1.23	(0.50, 15.74)	4 (20.0)
Dehulled maize	30	29(96.7)	2.11	(0.46, 302.72)	2(6.9)	11(37.9)	0.51	(0.46, 35.88)	1(9.1)
Beans	29	16(55.2)	4.57	(0.59, 81.96)	1(6.3)	8(50)	6.42	(0.82, 8.08)	6(75.0)

(vi)   Aflatoxin Contamination of Food Grains used in School Meals per District

Table 21 shows the distribution of aflatoxins contamination of food grains from schools in different districts of Kilimanjaro region. The overall median of aflatoxins contamination of food grains from schools in all districts was 2.046 (0.55-3.46) µg/kg and the highest median of 6.20 and range (0.46-438.53) µg/kg for total aflatoxin was found in a school at Same district, while the lowest median of 2.05 (0.49-31.25) µg/kg was found in samples from a school located in Moshi district. The highest concentration of 35.88 µg/kg for AFB1 was found in dehulled maize sample from a school in Same district while the lowest concentration of 0.44 µg/kg was found in rice sample from a school in Moshi District.

Table 21:   Aflatoxins contamination of food grains used in school meals per district

District	Number of sample	Aflatoxin total (µg/kg)			Aflatoxin B1 (µg/kg)		
		Positive samples n (%)	Median	Range	Positive samples n (%)	Median	Range
Hai	24	24(100)	2.45	(0.20, 100.65)	11(45.8)	0.89	(0.50,11.94)
Moshi	24	17 (70)	2.05	(0.49, 31.25)	5(29.4)	0.69	(0.44,6.08)
Mwanga	24	18(75)	2.47	(0.59, 81.96)	7(38.9)	1.29	(0.46,6.20)
Rombo	15	11(73.3)	2.05	(1.12, 179.67)	6(54.5)	1.04	(0.51,14.96)
Same	16	12(75)	6.20	(0.46, 438.53)	8(66.7.)	1.37	(0.46,35.88)
Siha	16	9(56.3.)	2.56	(1.96, 6.73)	4(44.4)	6.42	(1.14,6.73)

Table 22 presents total aflatoxins contamination in relation to the sources of maize, storage and preparations before cooking. Contamination was higher in purchased maize, with a range of 1.15-438 µg/kg in comparison to the maize produced by schools which ranged between 0.61-2.02 µg/kg. Likewise, maize stored in the ordinary sacks had higher contamination 1.15 - 438.52 µg/kg than its counterpart maize stored in silos (0.61-35.85 µg/kg). Moreover, maize samples stored without pesticides application had high contamination levels ranging from 0.61-438.53 µg/kg.

Table 22: **Aflatoxins contamination based on the sources, storage and preparation before cooking**

Variable	Number of samples (n)	Number of positive samples n (%)	Total aflatoxins contamination (µg/kg)	
			Median (µg/kg)	Range (µg/kg)
Source of maize				
Purchase	24	24(100)	4.37	(1.15-438.52)
Own production	6	5(83.3))	1.20	(0.61-2.02)
Storage				
Ordinary Sacks/bags	16	12(75)	5.71	(1.15-438.53)
Silos and sacks/bags	14	6(42.8)	2.02	(0.61-35.85)
Preparation				
Commercially purchased maize flour	11	11(100)	2.62	(1.15-438.53)
Winnowing and sorting	12	12(100)	3.48	(0.61-179.67)
Winnowing and decortation	7	6 (85.7)	5.59	(1.85-12.17)
Control of pests				
Application of pesticides	6	5(83.3)	11.35	(1.96-35.85)
No pesticides application	24	24(100)	2.61	(0.61-438.53)

(vii)   Assessment of Dietary Exposure to Aflatoxins

Table 23 presents aflatoxins exposure among adolescents through consumption of susceptible food ingredient as sole or part of meal. The highest exposure to total aflatoxins ranged between 0.70-973.45 (median 17.25) ng/kg/bw/day while that of AFB1 ranged between 0.05-81.06 ng/kg/bw/day (median 5.02) ng/kg/bw/day due to consumption of maize flour as part of stiff and thin porridge. Rice had least exposure ranged between 0.37-1.99, median 0.72 and 0.02-0.08, median 0.04 for total aflatoxins and aflatoxins B1, respectively. Margin of Exposure (MOE) for all (100%) adolescents ranged between 2.1 to 8500 which falls below the safe margin of 10 000 recommended by EFSA, 2007 indicating the risk of exposure to unacceptable levels of aflatoxins through school meals (Table 23).

Table 23:   **Dietary exposure to aflatoxins due to consumption of susceptible food**

Type of food	Total aflatoxins (ng/kg/bw/day)		AFB1(ng/kg/bw/day)		Margin of Exposure
	Median	Range	Median	Range	% of adolescents with exposure > 0.017 ng/ kg/ bw/ day
Maize flour	17.25	0.70- 973.45	5.02	0.05-81.06	100 (n=89)
Dehulled maize	4.48	0.36- 26.67	0.87	0.16-3.29	100 (n=126)
Rice	0.72	0.37-1.99	0.04	0.02-0.08	100 (n=61)
Beans	12.67	3.29- 67.60	6.40	0.28-66.04	100 (n=155)

(viii)   Knowledge and Awareness of Aflatoxins Contamination of Food Crops

Less than half of the respondent staff 14 (45.1%), reported to have heard about aflatoxins mainly from media 11(78.5%) while few learnt from the internet and school during their schooling 3(21.4%). Of the 14 respondent staff, only seven 7 (50%) had some knowledge on ways to control aflatoxins contamination of food crops. Out of them, 6 (85.7%) mentioned the use of proper storage to prevent fungal growth and 1 (14.3%) mentioned washing and dehulling of maize. Moreover, seven staff mentioned health effects caused by aflatoxins, out of which cancer and death were mentioned by 3 (43.0%) while diarrhea and vomiting were mentioned by 4 (57.1%) of the respondents' staff. On the other hand, out of 164 students, only 20 (12.2%) were aware of aflatoxins through media 16 (80.0%), school, internet and other sources 4 (20.0%); while 144 (87.8%) reported to have never heard about aflatoxins. Of the students who were aware of aflatoxins, 5 (25%) were able to mention aflatoxins control measures and two students were able to mention causes of aflatoxins (Table 24).

Table 24: **Knowledge and awareness of aflatoxins among staff responsible with school meals (n=31) and students (n=164)**

Variable	Staff n(%)	Students n(%)
Awareness of Aflatoxins		
Yes	14 (45.2)	20(12.2)
No	17 (54.8)	144(87.8)
Source of information		
Media	11(78.6)	16 (80.0)
School and internet	3(21.4)	4(20.0)
Know aflatoxins control measures		
Yes	7(50.0)	5(25.0)
No	7(50.0)	15 (75.0)
Know causes of aflatoxins		
Poor storage and fungus	7(50.0)	2(40)
Don't know	7(50.0)	3(60)
Known ways to control aflatoxins		
Proper storage to prevent fungal growth	6(85.7)	2(40.0)
Washing and dehulling	1(14.3)	
Don't know		3(60.0)
Known health effects caused by aflatoxins in human		
Cancer and death	3(43.0)	1(20)
Diarrhea and vomiting	4(57.1)	4(80)

4.1.4 Risk of Pesticide Exposure among Adolescent in Boarding High Schools

(i) Method performance and quality assurance

The mean percentage recovery for Cypermethrin, Chloropyrifos, Pirimiphos methyl, Permethrin, Deltametrhrin and Gamma cyhalothrin pesticide ranged between 93 to 113% which indicates the results were within the acceptable range (SANCO, 2012). The limit of detection was 0.002 µg/g for Cypemethrin and Gamma cyhalothrin and 0.003 µg/g for Chloropyrifos, Pirimiphos methyl, Permethrin, and Deltametrhrin. Limit of quantification was 0.01 µg/g for all pesticide measures with retention time ranged between 12.36 to 26.95 minutes. The coefficient of correlation (r^2) ranged between 0.09964 to 0.9999 showing a good linearity of the method (Table 25).

Table 25:   **Method performance and quality assurances**

Pesticide	Retention time	LOD µg/g	LOQ µg/g	r^2	Mean% recovery
Cypermethrin	12.45	0.002	0.01	0.9964	113
Permethrin	23.03	0.003	0.01	0.9987	96
Pirimiphos methyl	12.36	0.003	0.01	0.9999	102
Chloropyrifos	13.11	0.003	0.01	0.9992	93
Deltametrhrin	26.95	0.003	0.01	0.9998	98
Gamma cyhalothrin	21.66	0.002	0.01	0.9988	110

(ii)   Pesticide Residues in Food Grains used for School Meals

Table 25 presents results for pesticide residues in samples of maize and beans used for school meals. The findings indicated that, for all tested samples pesticide residues were below the respective limit of detections indicated in Table 26.

Table 26:   Pesticide residues in food grains used for school meals

Sample	Pesticide					
	Permethrin mg/kg	Deltamethrin mg/kg	Cypermethrin mg/kg	Gamma–cyhalothrin mg/kg	Pirimiphos methyl mg/kg	Chloropyrifos mg/kg
Beans (n=30)	BDL	BDL	BDL	BDL	BDL	BDL
Maize flour n=30)	BDL	BDL	BDL	BDL	BDL	BDL

*BDL – below detection limit

4.2 Discussion

4.2.2 Food Consumption in Boarding Schools

Findings show that adolescents diet in boarding high schools in Kilimanjaro region was confined with traditional starchy staples mainly cereals accompanied by legumes. The usual meal was mainly maize and kidney beans and less consumption of other starchy staples like roots, tubers and plantains. Moreover, animal source food, micronutrients rich fruits and vegetables were less consumed hence the meals are not balanced and whatever is provided lacks diversity. Similar findings were reported in school adolescents from Ghana and Ethiopia (Gali *et al*., 2017; Abizari *et al*., 2019).

Boarding schools' menu in this study were repetitive with fixed dishes in each day mainly maize based thin porridge (*Uji*) and stiff porridge (*Ugali*), beans, dehulled maize - bean meal (*Kande)* and rice making it very monotonous and giving adolescents no other food choices as they consume whatever the school provides. Stiff porridge, traditionally made from maize flour was the most consumed and usually accompanied with kidney beans. Maize accounts for 80% calories and 35% proteins intake in majority of the population in Tanzania (Mtaki, 2019). It is grown in many parts of the country thus increasing access to home-grown food, relatively cheap and affordable compared to other cereals such as rice and wheat (Minot, 2010) which are grown in selective parts of the country, relatively expensive and only affordable to a certain class of the population.

Monotonous diets are generally deficient in one or more essential nutrients hence increase the risks of malnutrition (Korkalo, 2016; Nicholaus *et al*., 2020). The quality and quantity of school meals are very essential to meet the nutritional needs of the adolescents (Erkan, 2011; Global Panel, 2015). Provision of diversified diet in schools not only enhance healthy eating but also food choices which enable adolescents to establish healthier dietary behavior and contribute to the prevention of nutrition-related diseases/conditions later in life (Aliyar *et al.,* 2015; Meko *et al.,* 2015).

The study found that the average maize flour consumed by adolescents per day as part of stiff and thin porridge ranged between 51 to 638.2 g/person/day on dry basis while the amount of dehulled maize consumed was about 173.1 to 454 g/person/day. This consumption is higher compared to that reported among adolescents (10-17 years) and adults (60 years) in South Africa and Kenya which were 368 ± 10.3 g/person/day and 400 g/person/day, respectively

(Kilonzo *et al.,* 2014; Shephard *et al.*, 2007). On the other hand, diet rich in cereals and legumes are usually inadequate to meet micronutrients requirements (Miller *et al.*, 2013). Furthermore, cereals and legumes contain a high amount of phytates which are inhibitors of micronutrients absorption such as iron and zinc which are essential for adolescents growth and development (Erkan, 2011; Gibson *et al*., 2010; ; Makori *et al.*, 2017).

4.2.3   Nutrients Intake among Adolescents

Findings show that adolescents had energy intake below the RDA. This was consistent to the findings of a studies from Kilosa rural adolescent (Maziya, 2014), India (Parimalavalli & Sangeetha, 2011) and Zambia (Bwalya, 2015) which reported low energy intake among adolescents. However, the adolescents' tendency to underreporting/misreporting food intake might have contributed to the underestimation of energy intake (Livingstone *et al*., 2004). The intake of proteins and carbohydrates were slightly higher above the RDA. This has been contributed by high intake of kidney beans and maize based food as the main sources of proteins and carbohydrates in boarding schools. Male participants had a significant high intake of proteins and carbohydrates as compared to their female counterparts ($P< 0.001$). The difference is more related to the portion size consumed between male and female adolescents. Even so, the consumption of proteins was observed to be higher but the proteins sources were mainly from plants which jeopardize the interpretation of these findings in terms of quality of proteins consumed. Findings show that there was minimal consumption of animal source proteins such as meat. Others sources such as fish, milk and eggs were missing in schools' menu. Animal sources proteins have been classified as good quality proteins due to the presence of essential amino acids which are easily digestible and bio available in the body (Boye *et al*., 2012), animal proteins are good source of essential micronutrients (Phillips *et al.*, 2015). However, animal source proteins are considerably expensive hence most of the boarding schools do not afford its regular consumption hence the reason for excluding them completely from the school menu.

Fat intake among adolescents in this study was slightly higher above RDA. These findings were inconsistent with findings from Nigeria and Kilosa which reported low dietary fat intake among adolescents (Deka *et al*., 2015; Maziya, 2014). In line with this research, studies from Spain, South Africa, Benin and Nigeria reported high fat intake among adolescents (Anyika *et al*., 2009; Hilsen *et al.,* 2010; Nago *et al.,* 2010; Serra-Majem *et al.,* 2000; Venter, 2010). Adequate intake of fat acts as a source of energy, enhances the absorption of fat-soluble

vitamins and it is an essential component of cell membrane and certain hormones (Aranceta & Pérez-Rodrigo, 2012). Moreover, fat improves the palatability of food (Anguah *et al*., 2017). The WHO recommended that less than 30% of the daily energy must be from fat (WHO, 2018). On the other hand, high intake of fat during adolescence is associated with increased risks of overweight and obesity hence increase risks of nutrition-related diseases later in life (Das *et al*., 2018). Findings from the study found that cooking oils added to school meals and bread spreads which students purchase on their own were the main sources of fat. Significantly, female had higher fat intake than male which was contributed by the consumption of high-fat food such as margarine and peanut butter. Female' adolescents regularly enhanced the flavor of their meals by adding peanut butter and margarine particularly in maize thin porridge (*Uji*) and maize and bean meal (*Kande*).

Micronutrients adequacy during adolescence is essential not only to support adolescents' health status but also the future health in adulthood (Nicholaus *et al.*, 2020; Soman *et al,* 2017). The increased demand for micronutrients during adolescence is contributed by growth spurts and hormonal changes (Erkan, 2011). Micronutrients deficiencies during adolescents can cause impaired growth, delayed sexual maturation and poor reproductive outcomes later in life particularly among girls (Iglesia *et al*., 2010). Findings in this study indicated that micronutrients intake such as calcium; zinc, iron and vitamin C were below RDA for both sexes. Generally, inadequate intake of calcium, zinc and iron was due to a low intake of animal sources food. In this study, none of the study participants had sufficient intake of calcium. Adequate calcium intake is important for bones development during adolescence and for prevention of osteoporosis in adulthood (Erkan, 2011; Lytle, 2002). About 45% of adult bone mass development occurs during adolescence (Iglesia *et al*., 2010).

Study findings show that iron intake was below RDA among studied adolescents. Consistent to these findings are reports from India and Ethiopia which showed that school going adolescents had low iron intake due to high consumption of non-haem rich food and food that inhibit the absorption of iron (Deka *et al.*, 2015; Teferi *et al.*, 2018). It is well known that, adequate iron during adolescents is important for building iron stores and maintenance of hemoglobin concentration, cognitive ability and muscle cells (Erkan, 2011). Inadequate intake of iron may lead to anemia which may affect adolescents growth and development (Sarah & Amanda, 2015).

Findings indicated that adolescents in private schools had low iron intake compared to their counterparts. It was expected that adolescents from private schools could have a better iron intake as compared to public' schools because private schools' adolescents are from the affluent families and are expected to have better meals from their schools, but this was not the case in this study. Even though the economic status of the individual adolescent was not studied, the differences in fees between private and public schools gives a hint on the family economic status. In this study there was no differences in food consumed by adolescents between private and public schools.

Another nutrient of interest was Vitamin C, this is an antioxidant which helps in prevention of non-communicable diseases such as some cancers and cardiovascular (Moretti *et al.*, 2014). Also, vitamin C is an important enhancer in the absorption of non-haem iron in the body (Moretti *et al.*, 2014; Resmi *et al*., 2017). Majority of adolescents in the study population were below RDA for vitamin C. Sources of vitamin C for boarding scholars were limited, fresh oranges were observed to be the main source. However, oranges were rarely consumed due to seasonality and availability plus the fact that one has to purchase, in some schools it is not part of school meals even during the high season. Other sources like green leafy vegetables though rarely consumed but most of the time are overcooked as they were mixed with beans hence vitamin C might be lost during cooking. Traditional cooking methods lead to 90% loss of Vitamin C in the leaves and 50-60% is lost in cooking water (Prabhu & Barrett, 2009). Vitamin C is water soluble and easily degraded by high temperature (Santos & Silva, 2008). The amount of fruits and vegetables consumed by adolescents was too small to contribute significantly to micronutrients intake.

4.2.4 Adolescents Nutrition Status

Findings of this study revealed the co-existence of under nutrition and over nutrition among boarding school adolescents. The prevalence of overweight was 25.0% and obesity was 6.1% among boarding school adolescents. Consistency to these findings, another study conducted in Kilimanjaro region in 2020 reported prevalence of overweight among school adolescents was 23% (Tengia-Kessy *et al.,* 2020). However, the prevalence of overweight in this study is relatively higher than 12.7% reported in school adolescents in Babati (Tluway *et al.*, 2018). Findings show that the prevalence of overweight (30%) in female was higher than male adolescents (13.4%). Similarly, findings from a study in Ethiopia reported overweight in school adolescents 5.3% male and 16.2% female (Gali *et al*., 2017). Adolescent girls

overweight /obesity are attributable to hormonal changes that influence the accumulation of fat mass in adolescent girls than boys (Todd *et al.*, 2015). Moreover, male adolescents are more physically active compared to female (Al-Hazzaa *et al.,* 2012) and sometimes boys in boarding schools are engaged in vigorous activities including various sports which make them burn fat and build muscle mass. Also, there is an existing perception among the African community that fatness with round body shapes among female is associated with beauty hence female adolescents do not feel vulnerable due to overweight (Keding *et al*., 2013).

The higher prevalence of overweight and obese among adolescents in this study is alarming; In comparison to previous studies done in primary schools in Dar-es-salaam, there is an increase in the prevalence of overweight and obesity among adolescents in Tanzania. Some studies reported 9.8%, 15.9% overweight and 5.2%, 6.7% obese, respectively among primary school going children (Muhihi *et al*., 2013; Pangani, 2016). However, at national level prevalence of overweight and obesity among adolescent girls (15-19 years) stand at 10.6%. and 1.9%, respectively (MoHCDGEC *et al* ., 2019). Kilimanjaro region is among the regions with highest prevalence of overweight and obesity (MoHCDGEC *et al*., 2019).

On the other hand, the prevalence of underweight (1.2%) and stunting (3.7%) reported in this study was low compared to that reported in Ethiopia among school-going adolescents with underweight (13.4%) and stunting (3.6%) (Teferi *et al.*, 2018) and in Nigeria 6.4% underweight and 1.8% stunting (Adesina *et al.,* 2012). Adolescents stunting is a reflection of chronic malnutrition which resulted from prolonged poor nutrition, infection and environmental stress accumulated from fetal through adolescence (Christian & Smith, 2018).

Anemia was prevalent among boarding school adolescents. From the study, the overall prevalence of anemia among adolescents was 23.1%. Female adolescents had a higher prevalence of anemia 18.9% compared to 4.3% male. This was consistent with findings reported in Temeke district in Tanzania which indicated that adolescents' anemia 14.5% and 7.9% female and male, respectively (Massawe *et al*., 2002). The prevalence of anaemia in this study is lower than that reported in India 62% female and 46.0% male (Soman *et al.,* 2017) and Saudia Arabia 34.2% female and 16.7% male (Alquaiz *et al.*, 2015). Anemia causes are multifactorial but mainly is due to iron deficiency (Wilunda *et al.*, 2013). It is well stipulated that 50% of anemia cases are caused by iron deficiency (Stevens *et al.*, 2013; WHO, 2011). Iron intake in adolescents may be poor due to inadequate intake as a result of changes in dietary habit and insufficient iron in the diet due to poor bioavailability (De Andrade *et al.,* 2014). Moreover, the prevalence of anemia in adolescents could also be due

to physiological changes in both female and male adolescents (Erkan, 2011). The low prevalence of anemia in male adolescents could be attributed by the increase in hemoglobin concentration contributed by sexual maturation and reduced requirements occurred after the growth spurt (Alquaiz *et al.,* 2015). Likewise, the higher prevalence of anemia in female is due to rise in iron requirements due to menstruation (Erkan, 2011; WHO *et al.,* 2013). Anemia in adolescents has consequences on their growth and development, cognitive ability hence affects academic performances and irregular menstrual cycle in female (Onabanjo & Balogun, 2014). It also affects physical fitness, work productivity and may lead to reproductive complications (De Andrade *et al.,* 2014). Therefore, anemia especially in female' adolescents if not timely managed may be a source complication in the entire female' life cycle hence intergenerational malnutrition (Alquaiz *et al.*, 2015).

4.2.5 Knowledge and Awareness on Diet Related Diseases

Adolescence is a window of opportunity where most dietary and lifestyle behavior are developed. These can either be negative or positive behaviour which can affect their current nutrition and health status and increase their risk for development of diet related diseases later in life (Nti *et al.*, 2012). Most of the diet- related diseases result from poor eating habits. Findings, from this study show that adolescents and staff responsible for school meals were aware on diet related diseases. Staff and students were able to mention some diseased related to diet signifying they are aware of the diseases/conditions. However, the   awareness on diet related diseases could have been obtained through school learning.

Similarly, adolescents' girls (55.8%) in Ethiopia had good knowledge on diet related information which was obtained from schools (Melaku *et al.,* 2017). Basing on their responses, both staff and students had low and fair knowledge on chronic diet related diseases like obesity, diabetes, cancer and hypertension. This is possibly contributed by insufficient nutrition and health education offered in schools in Tanzania (Borge *et al.*, 2008). Moreover, in some communities some of the diet related conditions for example obesity are not regarded as health problem rather a sign of wealth and good living hence people could not perceive it as threat to health (Ziraba *et al.,* 2009). In a study conducted in a study among adolescents and children in Nigeria found that 23.4% indicated that obesity is not a health problem (Fagunwa, 2021). A low knowledge on chronic diet related diseases among high school adolescents was reported in Ghana (Nti *et al.*, 2012).

4.2.6 School Meals and Aflatoxins Exposure

Boarding school scholars including adolescents are major consumers of cereals and cereal products mostly accompanied by legumes; however, the safety of school meals has received less attention in developing world (FAO & WHO, 2019). Of the grains used in these schools, little is obtained through own production, and the larger percentage purchased from within the Kilimanjaro region and occasionally purchased outside from the nearby regions such as Arusha, Tanga and Manyara. In Tanzania, cereals especially maize and maize products which are considered staple from various geographical locations have been widely reported to be contaminated by aflatoxins and sometimes in conjunction with fumonisins (Kamala *et al.*, 2015; Kimanya *et al.*, 2008; Magoha *et al.*, 2016; Shirima *et al.*, 2013). However, consumption of aflatoxin highly contaminated maize has been associated with the aflatoxicosis occurred in Dodoma and Manyara region in 2016 where 67 people were affected and 14 death (Okoth, 2016). In addition to these, cereals and cereal based products are susceptible to other mycotoxins such as ochratoxins, zearalenone, deoxynivalenol (Geary *et al.*, 2016; Kamala *et al.*, 2015; Kimanya *et al.*, 2014), however, these later cases have received less attention due to resource limitations and lack of strong surveillance systems. The occurrence and co-occurrence of mycotoxins in staple cereals can double the risk of human exposure especially when they are consumed monotonously and in large quantities (Kamala *et al*., 2017; Palumbo *et al.*, 2020). Moreover, food from subsistence farmers and local markets/traders in rural areas are rarely inspected. Likewise, maize processing industry in Tanzania is dominated by small scale millers who obtain maize from subsistence farmers and local traders, and subsequently these maize are milled into flour which lacks real traceability on the final products (Wilson & Lewis, 2015). This lack of strong food safety monitoring and regulatory systems can present a serious health risks to consumers. In recent past, Kenya has banned five Kenyan maize flour brands from commercial maize millers due to presence of unacceptable levels of aflatoxins (Mwinzi, 2019). In early March 2021, the country through its Agriculture and Food Authority also banned maize importation from its neighbor countries Tanzania and Uganda, claiming that their food safety surveillance program has revealed high levels of mycotoxins in maize from the two countries (Eastern Africa Grain Council [EAGC], 2021).The authority reported that the levels have been consistently beyond safety limits, and therefore unfit for human consumption (EAGC, 2021).

4.2.7   Food Grains Handling in Boarding Schools

Majority of the studied schools store their food grains in polypropylene bags /sacks while few stored in metal silos. Polypropylene bags /sacks storage accelerates pest infestation which damages the grains and promotes fungal growth (Shabani *et al.,* 2015), creating favorable condition for aflatoxins production. However, due to inadequate knowledge on biological and environmental predisposing factors that promotes mycotoxins contamination and compromise the safety of food, schools still use ordinary polypropylene sacks for storage of cereals. Though storage of grains in ordinary bags/sacks were more contaminated compared to those stored in silos, interpretation of this finding was confounded by fewer samples obtained from the later storage facility. Metal silo is considered expensive and unaffordable by majority of the studied schools. Moreover, 57.1% of the schools stored maize for more than six months after harvesting; Storage time may influence aflatoxins contamination especially when storage conditions are inadequate. Kos *et al.* (2018) found that contamination levels increased as the storage time of maize increases from three to seven months in a study conducted in Serbia. Likewise, Kaaya and Kyamuhangire (2006) found that most of the maize kernels stored from two to six months tested positive for aflatoxins as reported in a study conducted in three agro-ecological zones in Uganda. In another study conducted in Kongwa Tanzania, it was found that maize stored for 180 days were highly contaminated compared to those stored in less than 180 days (Sasamalo *et al.*, 2018).

4.2.8   Aflatoxins Contamination in Cereals and Legumes used for School Meals

Aflatoxins contamination levels found in maize flour and dehulled maize in this study were consistent with the findings reported by Makori *et al.* (2018) and Kilonzo *et al.* (2014) in Tanzania and Kenya, respectively. The high aflatoxins contamination in household maize and maize products ranged from 56.8- 427.8 µg/kg and 18-480 µg/kg, respectively. Similarly, a study in Burundi and Eastern Congo reported higher aflatoxins contamination in maize flour ranged from 2.5 to 350 µg/kg (Mutegi *et al.,* 2018). However, the levels of aflatoxins contamination of grains found in this study was lower than 588 µg/kg reported in Nigeria (Adetunji & Atanda, 2017). Kimanya *et al.* (2008) reported aflatoxins contamination levels of up to 158 µg/kg (median 24 µg/kg) in maize which is lower compared to the current study. Total aflatoxins level in dehulled maize in the current study is higher than in *Muthokoi,* a traditional dehulled maize in Kenya (Mutungi *et al.*, 2008 & Kilonzo *et al.,* 2014). Dehulling process removes seed coat, the embryo and the tip cap where the toxins are ingrained

(Karlovsky *et al.*, 2016). Dehulling reduced aflatoxins in maize by 92% (Matumba *et al.*, 2015). Likewise, Mutungi *et al.* (2008) reported aflatoxin reduction of 46% in *Muthokoi* However, the high aflatoxins content in dehulled maize in this study might be due to the fact that, the dehulled maize were re-stored in the original sacks for time to time use and thereby allowing re-contamination. Sprinkling of water on maize grains during dehulling might have increased moisture content of the grains creating favorable condition for re-contaminant fungal to grow and produce aflatoxins. Dehulled maize are usually cooked with kidney beans thereby reduces the proportion of maize in the pot. It is expected that, replacing part of maize with kidney beans in the pot goes hand in hand with reduced proportion of maize that would have been consumed if cooked alone and thus reduced chances of consuming larger amount of aflatoxins susceptible maize. Surprisingly, in this study, kidney beans were also contaminated with aflatoxins hence increases the possibility of multiple sources of exposure. Kidney beans are highly consumed as an accompaniment in almost all meals consumed in schools. Beans are considered less susceptible and hence rarely assessed for aflatoxins contamination, but due to its high consumption and the levels of contamination found in this study, it is suggested that attention should be directed on beans as well. The level of total aflatoxins in beans ranged from 0.59 µg/kg to 81.96 µg/kg and that of AFB1 ranged between 0.82 µg/kg to 8.08 µg/kg. Similar findings were reported in Zimbabwe whereby beans from small holder farmers from two districts were contaminated with aflatoxins at levels of 27.3 µg/kg and 70.9 µg/kg, respectively (Maringe *et al*., 2017). Beside, lower aflatoxins contamination levels in beans (3.0 µg/ kg, mean 2.49±0.11 µg/ kg) were reported in Babati District in Tanzania (Nyangi, 2014). Burundi and Congo in a range between 1.9 to 6.6 µg/kg (Udomkun *et al.,* 2018). Nonetheless, higher aflatoxins contamination in black beans with seed coat were contaminated (120.5 ± 24.1 µg/kg) and black beans without seed coat (115 ± 23 µg/kg) were reported in Costa Rica. Red kidney beans with and without seed coat were contaminated with aflatoxins at levels of 144.8 ± 29 µg/kg and 57 ± 11.4 µg/kg, respectively (Sancho *et al.,* 2015). In Nigeria, beans from market were reported to contain high levels of aflatoxins between 16.2 to 137.6 µg/kg (mean 59.29 ± 14.85 µg/kg) (Makun *et al*., 2010). Compared to other cereals, rice had the least aflatoxins contamination levels ranging between 0.20 µg/kg to 3.41 µg/kg (median of 2.01 µg/kg). This is in conformity with the findings from Kimanya *et al*. (2016) where relatively low aflatoxins levels (0.01-3.84 µg/kg, mean 1.19 µg/kg) in rice from Kilosa, Misungwi and Mbarari Districts compared to other cereals like maize.

The highest total aflatoxins and AFB1 contamination levels in maize flour and dehulled maize were from a school in Same District. Though contamination of maize flour and dehulled maize was found higher in purchased maize than those obtain from own production, the proportion of own produced maize samples was insufficient to make a valid inference. On the other hand, these high contamination levels could have been contributed by the weather especially to the own grown or locally purchased cereals. Same District is located in the semi-arid which experience prolonged drought resulted from climate change and variability (Hella *et al.*, 2014; Mongi *et al.*, 2010). High temperature, poor rainfall distribution together with persistent drought periods, especially inter-seasonal drought spell increase the problem of moisture stress to crops in the field making them weak and vulnerable to fungal infection and ultimately increased chances of aflatoxins production (Hella *et al.*, 2014; Matata *et al.*, 2019; Mongi *et al.*, 2010). Plant stress such as physiological stress, insects damage and environmental stress like high temperature above 28°C increases susceptibility of crops to fungal infection (Mahuku *et al.*, 2019; Probst *et al.*, 2014). This contamination may continue after crop maturation and when exposed to warm temperature and humidity, both in the field and storage (Probst *et al.*, 2014). Strong correlation between aflatoxins contamination and drought severity was reported by Hamidou *et al.* (2014).

The findings indicated that, out of the aflatoxins detected samples, 13.2% exceeded the maximum limits of 10 µg/kg for total aflatoxins set by the Tanzania Bureau of Standards (TBS, 2014). Maize samples were the most contaminated (32.1%). Likewise 12.1% of the samples exceeded maximum limit for AFB1.These findings concur with the report by Kimanya *et al.* (2016) whereby, maize and maize products were affected with aflatoxins above the acceptable levels. However, these findings are confined to schools in one region from one agro ecological zone and limited to seasonal variability, therefore may not be generalized to the entire calendar year and other agro ecological zones of the country, due to the fact that, aflatoxins contamination of crops varies by season and location. Nevertheless, occurrences of aflatoxins above regulatory limits in school food grains potentiate health risks to boarding scholars considering high frequency of consumption of the aflatoxins susceptible food.

4.2.9 Risk of Aflatoxins Exposure among Adolescents

Adequate and safe food is essential for provision of adequate nutrients to support rapid growth and development during adolescence, which is considered the heart of life course

(Das *et al.,* 2017).   Prolonged exposure to aflatoxins at the adolescent stage may be a potential risk factor to adverse health effects and poor performance in adulthood. The findings from this study show that, adolescents are highly exposed to aflatoxins in a range of 0.70-973.45 ng/kg/bw/day due consumption of maize flour as part of stiff and thin porridge. Moreover, consumption of contaminated maize flour *(ugali)* and beans meal lead to exposure ranged from 23.67 to 736.73 ng/kg/bw/day for total aflatoxins. This value is lower than the range of 1-180 704 ng/kg/bw/day following exposure to contaminated households maize kernel in Kenya (Kilonzo *et al.,* 2014). Additionally, the exposure level from this study was lower than 2167 ng/kg/bw/day reported in Nigeria (Adetunji & Atanda, 2017), but higher than 0.44 and 5.59 ng/kg/bw/day for a general adult population of Serbia, Croatia and Greece (Udovicki *et al*., 2019). Findings from this study, suggest immediate action in management of mycotoxins contamination of the routinely consumed maize, maize based products and beans, which are the threat to aflatoxins exposure in boarding scholars.

4.2.10 Knowledge and Awareness on Aflatoxins Exposure

The study found low level of awareness among teachers/ staff responsible for school meals and students, which indicate that, schools have been left behind in aflatoxins awareness raising campaigns. Kimanya *et al.* (2016) recommended on the importance of aflatoxins awareness raising campaign in schools both at primary and secondary levels. Low level of awareness and inadequate knowledge of mycotoxins contamination of crops and their associated health risks among subsistence farmers, local food traders and consumers results into negligence of efforts to their control and consumption of obviously damaged and susceptible grains (Liu & Wu, 2010). Similarly, low awareness and inadequate knowledge on mycotoxins to the farmers, cereal traders and consumers in three agro ecological zones in Babati, Kilosa and Chamwino Districts were reported (Suleiman *et al.*, 2017). In another study, where more than 50% of the participants had primary education, authors found a positive correlation between level of education to levels of awareness and knowledge on aflatoxins contamination of food crops (Kimanya *et al.,* 2016). In this study, secondary education level had no impact on the awareness of aflatoxins contamination of crop produce. Knowledge deficit and lack of awareness among stakeholders in Bukombe, Kongwa and Njombe Districts were also reported (Kimanya *et al.,* 2016). In addition, even care takers such as nurses had inadequate knowledge on aflatoxins. Low level of awareness on aflatoxins among farmers in Meru District was dependent on the level of education. The higher the level of education the higher the level of farmers awareness on aflatoxins contamination (Ayo *et*

al., 2018). Contrary to these findings, high level of awareness (78%) among farmers was reported in Ghana whereas majority did not consider aflatoxins as threat to human and animal health (Sugri *et al.,* 2015). Increase in awareness and knowledge of mycotoxins, their causes and control measures are thought to reduce mycotoxins contamination of cereals and other susceptible grains (Suleiman *et al.,* 2017) and ultimately protect human health.

4.2.11 Pesticide Applied in Grains used for School Meals

The application of pesticide in developing countries is widely used for grains before and after harvest to protect the grains from damage or loss (Manandhar *et al.*, 2018) that can be caused by pests. Grains require intensive use of pesticide during production and storage which may be found in grains or and in food prepared from grains (Grewal *et al*., 2017). During storage grains are treated with degradable pesticide such as organophosphate, synthetic pyrethroids and carbamate in order to prevent insect infestation and prolong the product storage (Grewal *et al.*, 2017). However, its effectiveness can be reduced over time due to degradation thus the active ingredient may change chemically and break down into products that may no longer have pesticide properties, thus decreasing the concentration of the original active ingredient (Grewal *et al.,* 2017; Mahugija *et al.,* 2017). In Tanzania reports indicated that, pesticides are highly used in food grains particularly maize and pulses (Lahr *et al.,* 2016; NBS, 2012).

The findings of this study indicated that few respondents used synthetic pesticides particularly insecticides commonly known as super actellic dust, Shumba, Shamba and Simba dust. These insecticides comprised of organophosphates and pyrethroids or combination of the two classes. On the other hand, some respondents reported the use of phosphine commercially known as phostoxin tablets to control insects' pests and rodents in the grain stores. Also rodenticide was used to control rodents. Nevertheless, majority of schools relied on purchased grains, which they were not sure if the grains were treated or not. Therefore, the cypermethrin, deltamethrin, permethrin, pirimiphos methyl, lambda–cyhalothrin and chloropyrifos pesticides were considered for analysis. The former four are common active ingredients of the common storage pesticide while the latter two Gamma–cyhalothrin and chloropyrifos were considered based on the previous studies done by Mahugija *et al.* (2017) and (Ndengerio-Ndossi *et al.,* 2005) which found chloropyrifos in maize and maize products. Essentially, these pesticides might have been applied before harvest to control insects and their residues may be found in the grains or in their products.

In a study conducted in Ulanga and Kilombero districts in Southern region of Tanzania found that among other pesticide, organophosphates and pyrethroids were the common pesticide sold to farmers indicating that they were highly used (Matowo *et al.,* 2020). Nonga *et al.* (2011) also found that most of the insecticides used in Northern region at lake Manyara basin were organophosphate and pyrethroids which are easily degradable.

4.2.12 Pesticide Residues in Cereals and Legumes used for School Meals

Findings from this study indicated that, pesticide residues of Cypermethrin, Deltamethrin, Gamma - cyhalothrin, Permethrin, Chloropyrifos and Pirimiphos - methyl in raw maize flour and beans samples used for school meals were below the respective limit of detections. Similar findings were reported in a study conducted in samples of cooked beans and stiff porridge in Dar-es-salaam which reported contamination below the method sensitivity (Ndengerio-Ndossi *et al*., 2005). Contrary to this findings, a study conducted in selected milling and markets in Dare-es salam and Ruvuma regions reported high concentration of pesticides up to 2220 and 2 µg/kg for organophosphate and pyrethroids, respectively in maize (Mahugija *et al.*, 2017). In a study conducted in Ghana, chlorpyrifos was detected at levels of 0.013 ± 0.004 mg/kg and 0.015 mg /kg in maize and cowpea respectively (Akoto *et al.,* 2013). The same study reported that the mean concentration of permethrin was 0.004 ± 0.002 mg/kg in maize and 0.001 ± 0.001 mg /kg in cowpea. Mean residual levels of ɣ- cyhalothrin detected were 0.028 ± 0.018 mg/kg in maize and 0.039 ± 0.032 mg/kg in cowpea (Akoto *et al*., 2013).

The low level of pesticides in school food might be contributed by the degradation of the pesticides due to prolonged storage time of the grains. Kaushik *et al.* (2009) indicated that pesticides residues are influenced by storage, handling and processing of raw agricultural commodities before consumption of the foodstuff. However, residues of the insecticides applied during storage of grains normally decline relatively slowly (Kaushik *et al.*, 2009). Schools normally obtained food from own production and purchases from local farmers and traders / markets. The former tend to store grains for about 3 to 12 months before the next harvest and they applied pesticides once per year before storage. Moreover, farmers and traders normally keep their grains till next harvesting season waiting for price increase (Bajwa *et al.*, 2014). The market price of the produces tend to increase within the six months of storage (Abass *et al*., 2014). Small holder farmers store grains for few months to a year before they are consumed or sold to the market where it might be kept further before being

purchased and used by other people (Bajwa *et al*., 2014; Manandhar *et al.*, 2018). The purchased grains would have taken longer time from the harvest to consumption and subsequently to when the residues were measured. However, during this period, pesticide residues in food commodities might have been reduced to undetected levels through natural degradation and the effects of temperature and long term storage (Ndengerio-Ndossi *et al*., 2005; Yigit & Velioglu, 2020). The stored wheat grains were treated with 12 mg/kg pirimiphos methyl and stored for 240 days. There was 40% reduction of the residues in the white flour compared to brans, and the whole flour (Sgarbiero *et al.,* 2002). Therefore, schools might have been used grains (maize and beans) that were previously treated and kept longer for the pesticides degradation to occur.

Nervetherless, some schools respondents reported the use of improved storage facilities such as metal silos that have contributed the infrequent application of pesticide in the stored grains. The metal silos maintain the quality of stored products, avoids use of insecticides and reduces losses significantly since the rodents are sealed off and insects suffocated (Abass *et al.,* 2014; Bwambale *et al.,* 2020).

In addition, processing removes the residues of pesticide in grains (Bajwa *et al.*, 2014). Sometimes residues may disappear up to 100% (Yigit *et al.,* 2020). The effects of food processing in removal of pesticides residues in food depend on the type of food, insecticides, nature and severity of processing procedure (Kaushik *et al.,* 2009). The maize milling process sometimes involves watering and removal of outer seed coat through dehulling and decortation. Some schools (35.5%) used commercially packed maize flour which were dehulled/decorted before milling. The low concentration of pesticides could be contributed by the fact that most of the pesticides are found in the outer part of the grain and removed by water and decortation/dehulling, which are preceding processes during milling. A study found a significant effect on residues when stored grains are milled, storage of wheat for 180 days after treatment with deltamethrin at 0.5 mg/kg where by the residues were reduced to between 0.03 and 0.2 mg/kg in the various forms of flour (Bolinova *et al.,* 2007). Mahugija *et al* (2017) reported low concentration of pesticides in polished maize flour (*sembe*) compared to unpolished maize flour (*dona*) in a study conducted in Dare-salaam and Ruvuma.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1   Conclusion

Diet of adolescents in boarding secondary schools is monotonous, predominantly maize and beans with minimal animal sources, fruits and vegetables which are important in the provision of key micronutrients. Moreover, boarding schools diet is inadequate in essential micronutrients such as iron, zinc, calcium and vitamin C Recommended Daily Allowance for adolescents according to age and sex. The co-existence of over nutrition and under nutrition in boarding schools found in this study is an alert showing the importance of routine nutrition assessment among adolescents. Considerably, maize, maize products and beans used for school meals were contaminated by aflatoxins, some to the levels above the national maximum limit. Concomitantly, adolescents are exposed to dietary aflatoxins to the levels that require public health attention. Though highly susceptible and monotonously consumed up to three meals per day, seven days in a week, these cereals are not assessed for aflatoxins contaminations, which might have increased the risks of adolescents' dietary exposure to these lethal toxins. The study also found out very limited awareness on aflatoxins contamination, their related health risks and management options among adolescents and teachers/staff responsible for school meals. The study showed pesticide residues in maize and beans used for school meals were below the detection limits. The findings are inconclusive considering that food samples were collected past six or more months after the harvesting time and were collected at one point in the value chain. However, the prolonged storage, milling and the use of improved storage facilities such as silos might have contributed to the reduction of pesticide residues in food grains.

5.2   Recommendations

This study calls for immediate intervention to improve school meals and reduction of aflatoxins contaminations in school grains. Monitoring of the adolescents' nutrition and health status is crucial and the health sector should take the lead in routine health and nutrition assessment. Schools are encouraged/ mandated to use adequate storage facilities that are rodent proof, aerated, clean, dry and regularly assessed to monitor moisture and temperature of the stored grains. Grains should be sorted and winnowed to remove bad grains before storage and food preparation. Training of farmers and grain handlers such as grain

traders and millers on good agricultural and management practices for reduction aflatoxins is necessary. Moreover, the current school adolescents are the future parents, farmers, and food traders; therefore, it is important that the responsible ministries and research institutions uphold aflatoxins knowledge and awareness campaign in schools, which are considered the best platform for knowledge dissemination. Concomitantly, quality dietary diversification of school meals is essential to enhance both adequate nutrients intake for growth and school performance and reduce aflatoxins exposure through predominantly maize and legumes diets. A routine risks assessment of this susceptible crops and enforcement of the regulation is a vital management strategy. A wide coverage cohort study focusing on school feeding, grains handling and the risk of exposure and co-exposure to mycotoxins and pesticide through consumption of school food alongside its actual exposure using biomarkers is recommended to detail the magnitude of the problem. Moreover, this research calls for a comprehensive study to asses' pesticide residues in the food grains throughout the value chain to determine the type of pesticide used during production and storage and to ascertain the risks at the consumption.

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APPENDICES

Appendix 1: Questionnaires for Boarding Secondary School Adolescents

Research title: Assessment of nutrition status and the risk of aflatoxin and pesticides exposure among adolescents in boarding secondary schools in Kilimanjaro Region

A	GENERAL INFORMATION	Fill in blanks/tick as appropriate	
1	Respondent Id/ Code		
2	School code		
3	District		
4	Ownership	1=Private 2= Public	
5	School system:	1= Girls 2= Boys 3= Co –education	
6	How long have you been in this school?	1=One year 2= two years 3=Three year	
7	Which year were you born?		
8	Height in cm---------------- cm Height2 in _____cm		
9	Body weight 1 in kg _____kg, Body weight 2 in kg _____ kg		
10	Hemoglobin concentration in mg/dL	mg/dL	
11	Date of data collection		
12	Education level of your mother	1=Illiterate 2= Primary 3=Secondary 4=Tertiary	
13	Education level of your father	1=Illiterate 2= Primary 3=Secondary 4=Tertiary	
14	What is the Occupation of your father	1=Employment 2= Farmers 3= Business 4=others (specify	
15	What is the occupation of your mother	1=Employment 2= Farmers 3= Business 4=Others (specify)	
16	**DIETARY PRACTICES**		

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17	How many times do you eat food per day when you are at school	1= Once 2= twice a day 3=Thrice a day 4= More than thrice	
18	Is there a fixed meal schedule for each day	1=Yes 2= No	
19	**SOURCES OF FOODS OUTSIDE SCHOOL**		
20	Do you have access to other sources of food apart from school meals?	1=Yes 2=No	
21	If yes what type of foods sold in schools	1= snacks 2= main meals 3 = Both snacks and main meals	
22	If Yes why do you prefer foods from outside school	1= availability 2=Diversity 3= Nutrition 4= Good taste	

D: Food Frequency Questionnaires

29 What do you usually eat and drink and in which amount (*respondent should indicate the frequency of consumption by indicated numbers eg if a food product is consumed once per day write number 1.*

Food items	Frequency of consumption					Portion size Average Amount consumed (per meal)	Sources of foods 1-=School meals 2= not in school meal
	Daily 1, 2, 3	Weekly 1, 2 ,3	Monthly 1, 2, 3	rarely √	Never √		
Cereals and cereal products							
Maize stiff porridge (ugali)							
Maize thin porridge (Uji)							
Boiled maize							
Maize grits (Makande)							
Sorghum stiff porridge							
Sorghum thin porridge							
Finger millet porridge							
Boiled rice							
Vegetable rice(pilau)							

White bread/sconces						
Chapati						
Vitumbua						
Burns						
Breakfast cereals						
Others(Specify)						
Roots, tubers, Plantains						
Irish potatoes boiled						
Fried potatoes and French fries						
Green bananas stew						
Sweet potatoes ,Yams						
Cassava						
Others (Specify)						
Legumes						
Beans stew						
Mixed dishes with beans (corn, bananas, rice potatoes)						
Peas stew						
Cowpeas stew						
Green grams stew						
Others (specify)						
Meat, poultry fish, eggs						
Beef stew						
Mixed dishes with beef (Pilau, banana stew,						
Goat						
Pork						
Liver including Chicken liver, goat						
Chicken sausages						
Eggs boiled						

Fried eggs						
Fish						
Sardines						
Others (Specify)						
Oils and fat						
Groundnuts						
Cashew nuts						
Vegetable cooking oil						
Margarine						
Peanut butter						
Others(specify						
Milk and milk products						
Fresh /fermented Cow's milk /						
Powdered milk						
Yoghurt						
Ice cream						
Others (Specify)						
Fruits						
Ripe bananas						
Water melon						
Mangoes						
oranges						
Pineapples						
Papaya						
Avocado						
Baobab						
Fruit juice						
Others (specify)						
Vegetable						
Cabbage						
Chinese						
Amaranth leaves						
Potato leaves						
Cowpea leaves						
Pumpkin leaves						
Kale leaves						
Mixed vegetables (salad)						
Cucumber						
Egg plants						
Pumpkin						
Tomatoes						

Others (Specify)						
BEVERAGE						
Tea without milk + sugar						
Milk tea+ sugar						
Coffee + sugar						
Milk Coffee + sugar						
Soy drink +sugar						
Chocolate drink +sugar						
Carbonated drinks (soda)						
Alcoholic beverage (Local brew, wine, beer						
SNACKS						
Biscuits						
Cakes						
Chocolates						
Popcorns						
Crisps (potatoes,banan as, yam, cassava)						
Sweets						
Others specify						
OTHER FOODS ITEMS						
Jam						
Honey						
Chill sources						
Pickles						

C: 24 hrs Dietary Recall

Please tell me everything you ate yesterday from the time you woke up in the morning until you went to sleep. Including all meals, snacks and drinks/beverage

Name of food (e.g. porridge, ugali, bread)	List of all ingredients used in preparation	Amount of food consumed (*Adolescent should give an estimate of the plate/cup/bowl*

D	KNOWLEDGE AND AWARENESS ON NUTRITION RELATED DISEASES	
17	Have you ever heard about nutrition related diseases?	1=Yes 2= No
18	If yes can you mention any three diseases which are associated with diet	1 2 3
19	What are the major causes of diet related diseases	1 2
E	KNOWLEDGE AND AWARENESS ON AFLATOXIN AND PESTICIDE CONTAMINATION IN FOOD	
20	Have you ever heard about Aflatoxin contamination	1=Yes 2= No
21	If yes where (specify)	
22	What are the causes of Aflatoxin contamination	
23	Can you mention the health effects caused by aflatoxins in human	
24	Do you know any effort used by school to reduce Aflatoxins contamination	1= yes 2=No
25	If yes can you mention them	
26	How long do you store grains used for school meals	
27	Where do you store grains used for school meals	1=Silo 2=Bags/sacks 3=Open space 4= I don't know
28	What do you do for the stored grains	1=Apply pesticides 2=No pesticides 3=Others(specify)
29	Do you know anything about pesticides residues on foods	1=Yes 2=No

Date_____

Principal Investigator's contacts; Email: nicholausc@nm-aist.ac.tz: Mobile No. 0759736820

Appendix 2: **Questionnaires for secondary school personnel responsible for school meals (Teacher/ staff responsible for school meals)**

A	GENERAL INFORMATION	Fill in blanks/tick / Circle as appropriate	
1	Respondent code		
2	School code		
3	District		
4	Ownership	1=Private 2=Government	
5	School system	1=Girls 2= Boys 3=Co education	
6	Date of data collection		
B. NUTRITION RELATED DISEASES KNOWLEGE AND AWARENESS			
7	Have you ever heard about nutrition related diseases?	1=Yes 2= No	
8	If yes, Can you mention any three diseases, which are associated with diet?		
C. SOURCES OF SCHOOL MEALS AND STORAGE PRACTICES			
9	Which grains do you use for school meals	1= Maize 2= Rice 3=Beans	
10	If the answer above is maize what are the main source of maize	1= own grown 2= purchases 3=others (specify	
11	If is rice what are the main sources of rice?	1= own grown 2= purchases 3=others (specify	
12	If is beans what are the main sources of beans?	1= Own grown 2= Purchases 3=others (specify	
13	If the source is own grown when did you harvest?		
14	If the source is through purchase when did you purchase?		
15	If purchased which season do you purchase grains	1=Rain 2=Dry 3= Others (specify)	
16	Do you purchase any of the school grains outside Kilimanjaro region?	1= Yes 2 = No	
17	If yes where(specify)		
18	Where do you store school grains?	1= Silos 2= Bags/sacks 3= Open space 4= Others specify	

19	How do you prepare maize to get flour for stiff porridge	1=Winnowing 2=Sorting 3=Washing 4=Soaking 5=Decortications 6=Dehulling 7=No preparation	
20	How do you prepare maize for makande (dehulled maize)	1=Winnowing 2=Washing 3=Dehulling 4=Sorting	
21	For the case of beans how do you prepare them before cooking	1=Winnowing 2=Washing 3=Sorting 3=Soaking	
22	For the case of rice how do you prepare before cooking	1=Winnowing 2=Washing 3=Sorting 3=Soaking	
23	How do you treat stored grains	1=Applying pesticides 2= No Pesticides 3= Others specify	
24	If you apply pesticides, which type?	1=Insecticides 2=Rodenticides 3= Fungicide 4= Others (specify)	
25	How frequent do you apply pesticides to the stored grains	1= Once per year 2= Twice per year 3=Thrice per year 4=More than trice	
26	Who is involved in the process of pesticides application once grains are purchased/harvested	1=Teachers 2= Students 3=Cooks 4= others specify	
27	Have the mentioned attended any training on pesticides application	1=Yes 2= No	
28	Do you purchase already treated grains	1= Yes 2= No 3 = I don't know	

F: DIETARY PRACTICES

29. What is your typical meals timetable for students in a week?

Day	Breakfast	Mid morning	Lunch	Supper
Monday				
Tuesday				
Wednesday				

Thursday		142		
Friday				
Saturday				
Sunday				
32. Do you have any guidelines for school meals?	1=Yes 2= No			
33. If No how do you plan your school meals?	1= Availability 2=Nutrition based Other (specify)			
KNOWLEDGE AND AWARENESS ON AFLATOXIN AND PESTICIDES CONTAMINATION				
34. Have you ever heard about Aflatoxins contamination?	1= yes 2= No			
35..If yes where (specify				
36.Do you have any method to reduce Aflatoxin	1=Yes 2=No			
37. Mention any two(2) health effects caused by Aflatoxin in human				
38..Do you know anything about pesticides residues in foods	1= Yes 2= No			

Appendix 3: Informed Consent Form for Teachers/Staff Responsible for School Meal

Research title: Assessment of dietary practices, nutrition status, the risk of exposure to aflatoxins and pesticide among adolescents in boarding secondary schools in Kilimanjaro Region

Purpose of the study: To assess dietary practices, nutrition status and aflatoxins and pesticide exposures among adolescents in boarding secondary schools in Kilimanjaro region. The study will be done by assessing dietary practices, nutritional status, aflatoxins and pesticide exposure among adolescents in boarding secondary schools.

Method and procedures: This study will involve taking measurements like height, weight and collecting blood samples from students. Also, samples of raw common consumed foods in school will be collected and the school dietary practices information will be collected from teachers/staff responsible for school meals and students.

Risks: There is no risk involved in the subject. The procedures are less invasive.

Benefits: There are no direct benefits from participation in this research. We hope that, in the future, other people might benefit from this study through improved school meals.

Confidentiality: Your information that is related to this study will be maintained confidentially and solely for the purpose of this study.

Your statement of consent and signature

To be signed by school teacher/staff responsible for school meal of the participating school

Please careful read the information below before completing and signing this consent form. If you have any questions concerning the study you are allowed to ask before signing.

I confirm that I have understood the study
I have agreed to participate in this study
I understand that participation of is voluntary and that I am free to be withdrawn from the study at any time without giving any reason
I know that records will be kept confidential and that I am free to be withdrawn from this study at any time.

Name   of   the   teacher/staff   responsible   for   school   meal
_____

School name_____ District _____
Signature_____ Date_____

Name of Researcher _____Date _____

Signature of Researcher _____Date _____

Appendix 4:   Assent Form for Students (Adolescents)

You are invited to participate in a research titled assessment of dietary practices, nutrition status aflatoxins and pesticides exposure in adolescents in boarding secondary schools in Kilimanjaro region.

Purpose of the study: To assess dietary practices, nutrition status and aflatoxins and pesticides exposure among adolescents in boarding public and private secondary schools in Kilimanjaro region.

Method and procedures: This study will involve taking individual measurements of height, weight and collecting blood samples from finger prick. Also small amount of raw food samples from school will be collected and the school dietary practices information will be collected from teachers and students.

Risks: There is no risk involved in the subject. The procedures are less invasive.

Benefits: There are no direct benefits from participation in this research. We hope that, in the future, other people might benefit from this study through improved school meals.

Confidentiality: Your information that is related to this study will be maintained in confidentiality.

Your statement of consent and signature

To be signed by the adolescents

Please careful read the information below before completing and signing this consent form. If you have any questions concerning the study you are allowed to ask before signing.

I confirm that I have understood the purpose of the study
I understand that participation is voluntary and that I am free to withdraw from the study at any time without giving any reason
I have understood that provision of blood sample is voluntary for the research
I know that your information will be kept confidential and that you are free to be withdrawn from this study at any time.

Name of the participant_____ District _____

School   name_____   Signature_____
Date_____
Name of Researcher _____Signature _____

Date _____

RESEARCH OUTPUTS

(i)   Publications

Nicholaus, C., Martin, H. D., Kassim, N., Matemu, A. O., & Kimiywe, J. (2020). Dietary practices, nutrient adequacy, and nutrition status among adolescents in boarding high schools in the kilimanjaro region, Tanzania. *Journal of Nutrition and Metabolism*, 2020, 1-14

Nicholaus, C., Martin, H. D., Matemu, A., Kimiywe, J., & Kassim, N. (2021). Risks of aflatoxin exposure among adolescents in boarding schools in Kilimanjaro region, Tanzania. *World Mycotoxin Journal*, *14*(2), 221-235.

(ii)   Poster Presentation