SAFETY AND QUALITY OF ORGANICALLY GROWN CLOVES (*Syzigium aromaticum)* AND BLACK PEPPER (*Piper nigrum l.*) IN TAWA WARD, MOROGORO-TANZANIA

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ABSTRACT

Participatory Action Research approach was used in this study involving organic spice farmers from Tawa Ward within Morogoro region, researchers from Sokoine University of Agriculture and Sustainable Agriculture Tanzania organization. Harvesting and post-harvest handling practices by clove and black pepper organic farmers in Tawa Ward were assessed. Five villages were visited, 107 organic farmers were interviewed, 34 among them inspected and provided samples for analysis. Fungal quality in association to post-harvest practices and moisture content was evaluated. Average moisture content was 18.7% (clove) and 12.3% (black pepper) in dry weight basis. It was observed that, farmers use experience and color change to determine moisture content and therefore fail to achieve the recommended moisture content of 12% as per Tanzania Bureau of Standards. Farmers in this study practices mixed farming in farm sizes of less than 5 hectares and harvest less than 100 kg of spices annually. However, poor drying and storage methods, mixing spice batches and attempts to treat spoiled spices were noticed as critical points that compromises safety and quality. Fungi were isolated by using spread plating method in PDA and V8 medias. Fungi identification were based on macro and microscopic characteristics. Black pepper samples were found to be contaminated by *Aspergillus, Rhizopus, Penicillium* and *Fusarium* fungal species at level of 2200 - > 30 000 cfu/g. Clove sample were contaminated by *Aspergillus* and *Rhizopus* species at level of 100-60°C fu/g in 46% of the samples. Longer storage time and mixing spices with previous batches during storage showed higher influence on level of fungal contamination. Also, this study measured levels of three heavy metals; Lead, Cadmium and Chromium in the spice samples. Levels of all three metals were found to be below permissible levels with average of 0.32±0.22mg/kg (Lead), 0.14±0.06mg/kg (Cadmium) and 0.33±0.16 mg/kg (Chromium). Detection of Lead in 10% of the sample was highly related to poor storage. This study recommends improvement in post-harvest handling approaches in order to ensure safety of the spices from Tawa Ward.

DECLARATION

I, BEATHA THOMAS MKOJERA do hereby declare to the senate of the Sokoine university of Agriculture that this dissertation is my original work, done within the period of registration and that it has neither been submitted nor been concurrently submitted for a higher degree award in any other Institution.

________________________________ ____________________

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The above declaration is confirmed by;

________________________________ ____________________

Prof. Bernard Chove Date

(Supervisor)

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DEDICATION

This work is dedicated to my beloved family.

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

′	Coordinate minutes
°	Degrees
°C	Degree Celsius
AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
BAM	Bacteriological Analytical Manual
CAC	Codex Alimentarius Commission
CFU	Colony Forming Units
E	East
EAC	East African Community
EFSA	The European Food Safety Authority
ESA	European Spice Association
FAO	Food and Agriculture Organization
HACCP	Hazard Analysis Critical Control Points
ICMSF	International Commission on Microbiological Specifications for Foods
IFOAM	International Federation of Organic Agriculture Movements
ITC	International Trade Centre
kg	Kilogram
NGO	Non-Governmental Organization
PDA	Potato Dextrose Agar
S	South
SAT	Sustainable Agriculture Tanzania
SUA	Sokoine University of Agriculture

spp	Species (plural)
SPSS	Statistical Package for the Social Sciences
TBS	Tanzania Bureau of Standards
TZS	Tanzanian Standards
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

At the global level, production of spices is estimated to be 6 000 000 tons per year (ITC, 2014). Tanzania had once (in 1999) ranked third by exporting 5% of Least Developed Countries' total spice exports. Sadly, Tanzania's share in the global spice trade dropped to 0.36% in year 2000 and it is estimated to have dropped further (ITC, 2014). Tanzania spice market lack consistent data on the quantities produced, sold and exported (ITC, 2014) and there is limited data on safety and quality of the produced spices (Temu, 2016). Unlike major cash crops, this was largely due to the absence of an official authority for the coordination of the production and marketing of spice crops (Akyoo and Lazaro, 2007).

However, recent trends indicate efforts and emphasis from government and donors on promoting spice production. In addition to government policy on common spices produced, the Board of External Trade (BET), which is under the Ministry of Industry, Trade and Investment (MITI) is nurturing the formation of a Spices Exporters Association (Caigher, 2004) further indication of increased government interest (Akyoo and Lazaro, 2017).

Due to the existence of variable but favorable climate and soil conditions a wide range of spice crops are cultivated in Tanzania. The average use of chemical fertilizers in Tanzania is less than 2 kg per hectare and per year (FAO, 2007) meaning that most land is never chemically fertilized. The most important spice crops produced for the local and export markets are clove, pepper, chilies, cinnamon, cardamom, ginger, coriander, vanilla, garlic, lemongrass and red onions. Most of them are organically produced in Morogoro, Arusha, Tanga and Zanzibar. Important spices grown in Morogoro include clove, pepper, cardamom and cinnamon, ginger, vanilla, and chilies.

Currently there is an increasing interest in spice organic farming. Rundgren (2008) and Bakewell-Stone (2006) estimates of the certified organic production land area in Tanzania to be over 80,000 hectares with at least 36 companies and 65 000 farmers involved in producing organically in the country. According to 2016 statistics, Tanzania is the sixths country in organic production worldwide and the third in Africa after Uganda and Ethiopia (IFOAM, 2018 as shown by Gowen, 2018).

However, only a small segment (less than 5% of spice output) is categorized organically Certified Spices in Tanzania. The production of organically produced spices is dominated by middle scale producers and processors. The rest of spice produced falls under Non-certified Spices produced by large number of small-scale producers and processors (ITC, 2014).

The market for organic products is relatively small but fast-growing and powered by consumers concern on food safety and healthy eating. In order to help small scale farmers who are major producers of organic crops, National Organic Agriculture Movements, often in cooperation with NGOs, are implementing guarantee schemes for smallholder farmers along the lines of Participatory Guarantee Systems (PGS) (NOAF, 2008).

While fertilizers and pesticides are not used in organic spice farming in Tanzania, other sources of food safety risk are facing the organic spice market. Drying is done while the produce is spread on mats laid on bare ground increasing the risk of microbial contamination and without overlooking chemical pollutant from water, air and soil (Akyoo and Lazaro 2008). Current status of spice value chain in Tanzania indicate that spices are dried without monitoring the moisture content during the process as well as the final moisture content, sales are done without sorting and grading, and purchase and export is still monopolized by middlemen traders. For example, cinnamon harvesting is done to barks that are more than five years of age. This hinder formation of cinnamon quills which are higher valuable products. Cinnamon is also dried without measuring moisture content and sales are done on undefined packaging (ITC, 2014).

There is very limited research and published basic data on safety and quality of organically produces spices in Tanzania which has seen to affect the existing market of organically produces spices (ProFound and Mugenyi, 2012; ITC, 2014) For example in 2001-2005 time period, Tanzania exported spices worth 609,051 Euros to European countries whereas data on such export was obtained and published by importing countries instead of Tanzania (Source: http://fd.comext.eurostat.cec.eu.int/xtweb (Eurostat) as reported by Akyoo and lazaro 2008). Also, limited research has affected the third-party certification system which is costly and hard to implement (Fouilleux and Loconto, 2017).

1.2 Literature Review

1.2.1 Postharvest handling of spices

The method and timing of harvest, handling, drying and storage have influence on the yield, quality and safety of the product. Harvesting of immature crops will result into small size, uncharacteristic shape and flavor/aroma and therefore, a poor quality. Early harvest also effects weight and price of the spices (Petropoulos *et al.* 2017).

Harvesting and processing methods of spices differ from one spice to another. Each spice requires individual expertise in its processing. Spices are produced from a large variety of plant parts such as rhizomes, barks, leaves, fruits, seeds, etc. However, there are some basic unit operations that are commonly applied to most spices. They include:

1.2.1.1 Harvesting and post-harvest treatments

Well matures spices have highest content of their active ingredients and are harvested manually or mechanically. Prior to drying several post-harvest treatments such as curing could be applied to improve color characteristics, lower transport and storage volume and accelerate the drying procedure (Das and Sharangi, 2018). Furthermore, for those convectional farmers, fumigation with ethylene oxide or other chemical treatments is applied to improve color characteristics and control microbial and insect infestations (Duncan *et al.*, 2017). However, due to humid and warm climatic conditions, it is important to have controlled harvesting and post-harvest treatments in order to reduce mycotoxin contamination that could occur during harvesting, post harvesting and storage (Jalili *et al*., 2010).

1.2.1.2 Drying of the raw materials

Drying is done to achieve a hygienically safe moisture level (around 10%). In tropical and subtropical regions, the plant material is commonly spread on the ground, sometimes on raised platforms or racks, and sundried for several days. Since the drying process is conducted under the open sun in the homestead, contamination due to dust and livestock is possible. Another important drying method applied is the use of stoves wherein open fire or heated iron pipes provide the heat for drying, even though the maintenance of uniform temperatures is difficult (Das and Sharangi, 2018). Several mechanical dryers have been developed to accelerate the drying process, to improve the hygienic conditions and to maintain constant drying temperatures within 45°C and 60°C in order to minimize the loss of volatile oils and discoloration, respectively. However, the use of both solar and artificial dryers is still scarce and natural sun-drying remains the most widely used method to small farms in developing countries (UNIDO and FAO, 2005).

1.2.1.3 Grading and size reduction

Grading is done to separate contaminants as well as damaged and discolored spices. Most spices are subjected to size reduction and graded to allow different forms of spices in the market, such as raw, dried or pre-ground dried (Balasubramanian *et al.,* 2016). Other characteristics used for grading include seed maturity, shape, size and color.

1.2.1.4 Packaging and storage

Dried spices in whole or in powdered form are usually packed into gunny packs and stored until they are delivered to the traders and the processing industry. Packaging provides ease of handling while maintain the characteristic quality of the product during postharvest distribution. It also adds an additional value to the product for marketing (Das and Sharangi, 2018).

Besides the drying operation, storage is another critical control point because the control of storage temperature and humidity is indispensable to prevent damages of the spice material. Susceptibility of dried spices to spoilage and toxin producing microorganism increases with water activity above safe limits, and storage temperature suitable for their growth (Duncan *et al.,* 2017). In many cases, small scale farmers store their produce under conditions favoring contamination by insects, rodents, and other vermin, thus increasing the risk for postharvest destruction and loss.

1.2.2 Problems Associated with Spice Production

1.2.2.1 Higher microbial loads and aflatoxin contamination

Microbial contamination causes up to 50% losses of spice harvest (White, 2002). Microbial load is accelerated by the hot and humid climate, the simple, unpretentious production conditions, extended drying times and often inadequate information to the farmers on hygienic and quality problems. The microbial populations are mainly composed of mesophilic and spore-forming bacteria, moulds and yeasts, among them pathogenic and food spoiling genera like *Salmonella, Clostridium, Bacillus, Listeria* and *Staphylococcus* (Tulu *et al.,* 2014; Elkhishin *et al.,* 2017). The growth of moulds creates serious problems mainly in paprika, chilies, coriander, nutmeg, ginger, and turmeric because of their mycotoxin producing potential. Mycotoxins, particularly the aflatoxins, exhibit pronounced heat stability therefore the most efficient ways to prevent mycotoxin would be and early inhibit of microbial growth (Akpo-Djènontin *et al.,* 2018)

1.2.2.2 Losses of valuable compounds due to endogenous enzyme activities, conventional processing and storage conditions

Metabolic activity of the plant material continues, and particularly deteriorative enzymes are still active after harvesting. The changes that occur include water loss, deterioration of flavor and color, softening and decay. Such changes may adversely affect color, taste and texture properties of the spices themselves but particularly of food products (Rodríguez-Verástegui *et al.*, 2016). Therefore, the immediate and complete inactivation of deteriorative enzymes is a prerequisite for the production of high-quality spices.

Sensitivity of spices active compounds, color and flavor to destruction and degradation increases during processing, storage, evaporation and oxidative reactions. Light and elevated temperature causes alterations in the genuine sensory profile of spices

(Schweiggert *et al.,* 2007). The poor stability of spice active compounds during storage and transportation is a serious economic problem because of the long time needed for the products to reach the consumer.

1.2.3 Spice production and supply by small scale organic farmers in Tanzania

Individual farmers who are primary producers are responsible for organizing harvesting and marketing of their crops (ITC, 2014). Others do so in small group associations that are formed to assist members collecting, processing and selling their produces. Medium scale farmers have their own collecting and processing centres. These do therefore undertake the harvesting and processing activities in more elaborately organized manner. For some spice crops harvesting is organized by middleman traders who buy crops in advance. Small scale farmers certified to grow spices organically have to sell their produce to medium scale organic farmers or buyers who facilitated their certification. It is also common for them to sell to middleman buyers of conventional types since there is no premium price offered to organic produces (Bakewell-Stone, 2006).

Value chain of organic spices faces marketing challenges due to lack of certification. The majority of spices are produced by small scale farmers in the rural areas. The recent need for certification of organically produced products has led to the categorization between organic and conventional spice farmers (Bakewell-Stone *et al*., 2008).

Benefits expected by organic spice farmers after attaining organic certification include premium prices, better yield from improved agricultural techniques and a guarantee to high value markets. However, for small scale organic farmers to achieve certification they must be prepared to incur costs related to the certified organic standard in the Tanzanian spice industry at all stages of production from land clearance to ultimate sale. In addition, direct costs may be incurred by farmers whilst attending organic agriculture seminars / training courses. Exporters on the other hand incur a countless of compliance costs ranging from farmer registration, record keeping, inspection, certification, field agency operation, farmer training, and premium price payment (Akyoo and Lazaro, 2007).

1.2.4 Organic food products standards and specifications

Most smallholder organic crops producers employ no clearly defined grades and standards (Jari and Fraser, 2014). Also, institutions for determining market standards and grades tend to be poorly developed in smallholder farmer's environments (Herman *et al.,* 2012). Therefore, this indicates that only well-organized farmers can benefit from trade liberalization by adopting strict quality control measures and obtaining the necessary certification for their goods (Baloyi, 2010).

Food standards are important tools to ensure food safety, to avoid consumer fraud and to enhance global trade activities (ISO/IEC, 2011). In April 2007, the East African Organic Product Standards (EAOPS), developed by a regional working group, were adopted by the member states of the East African Community (EAC): Uganda, Tanzania, Kenya, Rwanda and Burundi. The EAOPS is the first standard developed through a collaboration of private organic movements and national standard bodies and is the second regional organic standard in the World after the European Union's. Agricultural products from Tanzania that are labeled "organic" must therefore comply with these standards. The standard provides general requirements for organic production and specific guidelines for crop production, animal husbandry, beekeeping and instructions for handling and labeling of organic produce (EAC, 2007).

Other standard and requirements by organic producers include those made by Codex Alimentarius Commission, Tanzania Bureau of Standards and European Commission of Regulation. The European Spice Association (ESA), describes the quality minima for dried herbs and spices, which should be demanded by buyers when these products are purchased for further processing within the European Union (EC, 2006a).

1.2.5 Safety and health concerns related to spices

The safety of spices and dried aromatic herbs products highly depends on maintaining good hygienic practices along the food chain during primary production, storage, processing, packing, retail, and at the point of consumption. Codex recommends good hygienic practices at all points of spice value chain in the code of hygienic practice for spices and dried aromatic herbs (CXP/042e/, 2014). Spices are considered critical foods and attention needs to be paid to the application of spices as to ready-to-eat foods especially when they are not subjected to further heat treatments (Hertwig *et al.,* 2015).

Spore forming bacteria, including pathogens as well as non-spore forming vegetative cells of microorganisms have been found in dried spices. The safety of spices and dried aromatic herbs can also be affected by mycotoxin-producing molds. Van Doren *et al*., 2013 reported several outbreaks of illness associated with spice and seasoning consumption in Canada, Denmark, England and Wales, France, Germany, New Zealand, Norway, Serbia, and the United States. Such outbreaks resulted in reported human illnesses, 128 hospitalizations and two deaths. During the last decade of the 20th century, food-borne infections and intoxications due to spices have increased in several European countries (Székács *et al.,* 2017).

The complex supply chain for spices and dried aromatic herbs makes it difficult to identify the points in the food chain where contamination occurs, but evidence has demonstrated that contamination can occur throughout the food chain if proper practices are not followed. The unhygienic production conditions increase number of food-borne infections and intoxications (Székács *et al.,* 2017). Also, strong antimicrobial effects of spices (Babuskin *et al.*, 2014; Ghabraie *et al.*, 2016) challenge the task of detecting total microbial contaminations which potentially increase the risk for false-negative results (Lins, 2018).

Therefore, as regard to microbial contamination, compliance with technology specifications in cultivation, harvest and storage is of essential importance to avoid growth of pathogenic microbes and mycotoxins formations (Bosland and Votava, 2012). If properly dried at moisture level that is recommended by different standards for spices, spices do not support microbial proliferation as a result of their low water activity. However, some microorganisms, including pathogens and toxin producers, are able to survive in low-moisture foods. Microbial contaminants of spices include fungi, such as yeasts and moulds, as well as bacteria (Kara *et al.,* 2015; Kong *et al.,* 2014; Koohy-Kamaly-Dehkordy, *et al.*, 2013).

1.2.6 Microbiological standards of spices

Regarding microbiological food safety concerns in low-moisture foods, dried culinary herbs and spices including herbal tea were ranked in the top three (FAO/WHO, 2014a). Production standards addressing hygienic measures are set worldwide by various public and private institutions in order to prevent contaminations of culinary herbs/spices with microorganisms to unsatisfactory levels. Several guidelines that are specific for spices on good practices and HACCP are available in national, multinational and global level

(Schaarschmidt *et al.*, 2016). In addition to compliance with legal obligations, application of private standards is common and often requested by purchasers.

Microbiological criteria for spice products must be considered at all levels of production and supply chain. It might be difficult to establish control at all levels in developing countries, but official control should emphasize the assessment of spices as far as retail levels (Kantonslabor, 2012).

1.2.7 Heavy metal contamination in spices

Heavy metals are widely distributed in the environment and are released by natural (e.g. volcanic) activities and human (e.g. industry, military, agricultural) activities to air, water, and soil, from which they can be taken up by plants (Zhao *et al*., 2014; Laar *et al*., 2011). Even though soil represent a major sink for heavy metals to plant, metal uptake in crops is regulated by several factors, including bioavailability of the metal in soil, crop type and metal distribution in the crop (Tom *et al*., 2014).

Heavy metals comprise essential nutrients, such as Copper, Iron, Magnesium, as well as human toxic elements, which particularly applies to Arsenic, Cadmium, Lead, and Mercury (Jan *et al*., 2015; Tchounwou *et al.*, 2012) Arsenic, Cadmium, Lead, and Mercury have no identified physiological activity but have been proven to be harmful beyond certain limits (Nhapi *et al*., 2012) and rank highly among the priority metals with public health significance (Khillare *et al*., 2015).

Lead, Cadmium and Chromium have accumulative behavior and can produce health effects to human (Khan *et al.*, 2015). Despite its role in increasing enzyme activity and play part in carbohydrate metabolism (Al-Fartusie and Mohssan, 2017), Chromium is reported to harm biological systems when consumed in excess (Michalski, 2004; Costa and Klein, 2006; Wise *et al.,* 2016). Lead and Cadmium have no important functions in human body rather play toxic role to living organism, hence are considered as toxic elements (Lokeshwari and Chandrappa2006; Jarup 2003). Prolonged human consumption of foodstuffs contaminated with heavy metals may lead to the disruption of numerous biological and biochemical processes in the human body. Cadmium and Chromium act as carcinogens and Lead is associated with developmental of abnormalities in children (D'Mello, 2003; Wang and Du, 2013; Shadreck and Mugadza, 2013; Flora *et al.,* 2012).

Uses of spices and herbs have been increasing hence research works concerning them. Determination of heavy metal concentration on commonly traded spices is done in different countries worldwide (Soliman 2015; Bua *et al.,* 216.), but no study focused on heavy metal content of organic spices available in the Matombo Division. The widespread contamination of spices and herbs with heavy metals in last two decades has increased the scientific interest research as it has the harmful effect on human health (Inam *et al.,* 2013).

1.3 Problem Statement and Study Justification

Organic spice farmers in Tanzania face a challenge of certification to their produces. Processing certification for organic products is more expensive and require compliance to both organic and quality standards (Crowder and Reganol, 2015). In Morogoro, organic farmers who are working with Sustainable Agriculture Tanzania (SAT), benefit from training and market linking of their organic products. SAT buys 25% of their produces only when complies to the set specifications. Compliance to quality and safety standards and obtaining organic certification will expand their market. These organic farmers are partially aware of the chemical and microbiological safety required to meet certification standard. Data on the chemical contaminants and microbiological safety of their produces is lacking as they do not subject them to laboratory analysis. Also, these spice farmers have been experiencing color change, decrease in weight and presence of white dust during storage of dried spices especially black pepper.

This study assessed and documented compliance to food safety and chemical contaminants standard by organic farmers in Tawa Ward that is within Matombo Division in Morogoro region. Non-compliance with certified organic and safety standards has consequences for farmers, ranging from temporary to permanent exclusion from the organic scheme (Akyoo and Lazaro, 2008).

Implementing a certified organic system of production for Tanzanian spices may eliminate the requirement for special testing for hazards like pesticide residue levels. However, such tests together with other hazards tests like mycotoxins and heavy metals are mandatory for export market in the European Union (Curzi *et al.,* 2018). Organic spices are also expected to be microbiologically and chemically safe without compromising the organic integrity of their products. Along with compliance assessment of post-harvest practices, this study also assessed the microbiological and chemical safety of organic spices from Matombo Division. Microbiological assessment was mainly focused on moulds and yeasts, since these are associated with moisture levels and potential production of toxins that are difficult to eliminate postharvest.

Chemical assessment was focused on heavy metal analysis. Heavy metals have cumulative behavior and toxicity although they are generally present in agricultural soils at low levels (Chen *et al.,* 2016; Rodríguez-Bocanegra *et al.,* 2018). Heavy metal analysis provides profile of essential metals like Iron, Copper, Nickel and Zinc and also levels of toxic metals like Mercury, Lead and Cadmium.

Monitoring the levels of chemical toxicity and microbiological quality in organic spices would help ascertain the health impact of taking these spices and provide relevant data on organic spices in the country. Very little has been done to assess microbial status of spices in Tanzania (Temu, 2016) in comparison to the research and publication focused on staple crops like maize (Kamala *et al.,* 2015). Moreover, the generated data will also inform the consumers (buyers) of the status of the produce and assist them in decision making. Thus, will open doors to export market where the products could be imported into up market developed countries.

1.4 Objectives

1.4.1 General objective

To assess compliance of organic spices from Matombo to chemical and microbiological safety standards.

1.4.2 Specific objectives

The specific objectives of this study were to:

(i) assess harvesting and postharvest handling practices (drying and storage) of cloves and black pepper in order to establish the quality compromising stages

(ii) assess microbiological quality in relation to postharvest practices of cloves and black pepper from the study area focusing on isolation and identification of molds and yeast

(iii) assess levels of Lead, Cadmium and Chromium in Cloves and Black pepper from the study area

1.4.3 List of manuscripts

i. Assessment of harvesting and post-harvest handling practices on organically grown cloves and black pepper in TawaWard- Morogoro
ii. Fungal quality and identification of organically grown clove and black pepper related to their post-harvest handling practices.
iii. Lead, Cadmium and Chromium levels in organically grown clove and black pepper from Morogoro rural district

The findings of this research are reported in three manuscripts presented as chapters Two, Three and Four.

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CHAPTER TWO

Manuscript One

3.0 Assessment of Harvesting and Post-Harvest Handling Practices on Organically Grown Cloves and Black Pepper in Tawa Ward- Morogoro

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2.1 Abstract

Clove and black pepper are among the valuable spices, especially when grown organically. Quality and safety of spices highly depend on proper handling practices during and after harvesting. This study assessed harvesting and post-harvest handling practices by clove and black pepper organic farmers in Tawa Ward in Morogoro Rural District; in order to establish the quality compromising stages. A total of 107 organic farmers of such spices were interviewed and 34 among them inspected for their harvesting, drying, storage and other post-harvest handling practices. Spice samples were collected from inspected farmers for moisture content analysis. Freshly harvested spices had average moisture content of 60.3±9.2(clove) and 68.2±2.9(black pepper) whereas final dried spices had average moisture content of 18.9±3.4(clove) and 11.4±1.1 (black pepper). This study revealed that spice farmers use experience and color change to determine moisture content and therefore fail to achieve the recommended moisture content of no more than 12% as per TZS 30 2013; TZS 357 2012. Most farmers in this study practices mixed farming, with farm size of <5 hectors and harvest not more than

100 kg/year. However, poor drying and storage methods, mixing spice batches and attempts to treat spoiled spices were noticed by as critical points that could compromises quality of final spices. Treatment of spoiled and contaminated spices in order to retain the original color was highly related to farmers' unawareness on fungus and mycotoxins. Therefore, this study calls for intervention of improving farmers knowledge and make use of simple but effective methods to maintain quality of harvested organic spices. Drying of spices could be done on raised platforms instead of directly in the ground and making use of moisture meter to determine level of dryness along with storing spices in clean and dry places can be a good starting point on assuring quality of such organic spices is maintained along the value chain.

Key words: *spice harvesting, postharvest handling, drying, spice storage, clove, black pepper*, *Tawa Ward*

2.2 Introduction

Tawa Ward is found along the Uluguru Mountains within Matombo Division of Morogoro rural District. Altitude and weather of Tawa Ward favor growth of various spices including vanilla, cloves, black pepper, cardamom, cinnamon and turmeric. Majority of spice growers in this area are small-scale farmers and are considered to grow spices organically by default (ITC, 2014; Maerere and Van Noort, 2016).

Black pepper nicknamed 'king of spices' (Meghwal and Goswami, 2012) is a product of the mature fruits of *Piper nigrum L.*, a perennial woody evergreen climber. Under cultivation, pepper vines are trailed on supports and may attain a height of 20m or even more (Ravindran and Kallupurackal, 2012). In Tawa Ward; *Jatropacurcas*, Jackfruit, Kapok and Grevilea trees are used as trellising material for black pepper.

In some few cases big rocks, mango and coffee trees are also used to support the crawling of black pepper. More than 100 cultivars of black pepper are known (Ravindran *et al.*, 2000 a, b) but only three are cultivated in Tawa.

Clove, 'a champion spice' (Milind and Deepa, 2011) is one of the most ancient and valuable spices of the world used for culinary, pharmaceutical and perfumery purposes (Kendra, 2010). The clove of commerce is the dried aromatic fully grown unopened flower buds of the clove tree (*Syzigium aromaticum*) belonging to the family *Myrtaceae*. The clove tree is an evergreen tree, that grows to a height ranging from 8-12m and have large square leaves which begin to flower after six years of growth. Clove trees can vary based on shape of trees, bearing habits, cropping season, yield, color, shape and dimension of clove buds (Kendra 2010; Thangaselvabai *et al.*, 2010).

Harvesting and post-harvest concepts explained in this study are based on information obtained during field visits at the study area. The core of the study based on already existing well-structured focus groups. Total of 30 clove farmers, 77 black pepper farmers and 3 spice middlemen traders were directly involved in this study from five among the eight villages of Tawa Ward. The participants were interviewed, and their post-harvest handling practices were closely observed against the pre-prepared checklist.

Spice farmers in Tawa Ward under Matombo Division have been experiencing color change, decrease in weight and presence of white dust during storage of dried spices especially black pepper. The quality of clove and black pepper highly depends on maturity of harvested seeds, drying methods and storage conditions (Ravindran, 2017). Therefore, this study aimed to assess harvesting and post harvesting practices by farmers from five villages in Tawa Ward in order to establish the quality compromising stages so as to assist in the crafting of intervention processes.

2.3 Materials and Method

2.3.1 Study area

This study was conducted in Tawa Ward that is within Morogoro rural District. Five villages of Tawa Ward in Matombo Division were visited during the time of the study. Location coordinates and altitudes of the sites are shown in Table 2.1 and Fig. 2.1 below.

Table 2.1: Location description of the study area

Ward	Village	Latitude	Longitude	Altitude
	Uponda	07° 01.207' S	037° 46.637' E	218m
	Kisarawe	07° 00.755' S	037° 44.340' E	374m
TAWA	Milawilila	06° 59.758' S	037° 44.738' E	377m
	Kifindike	07° 02.367' S	037° 45.210' E	380m
	Tawa-center	07° 00.921' S	037° 43.878' E	395m

Source: Latlong, 2019

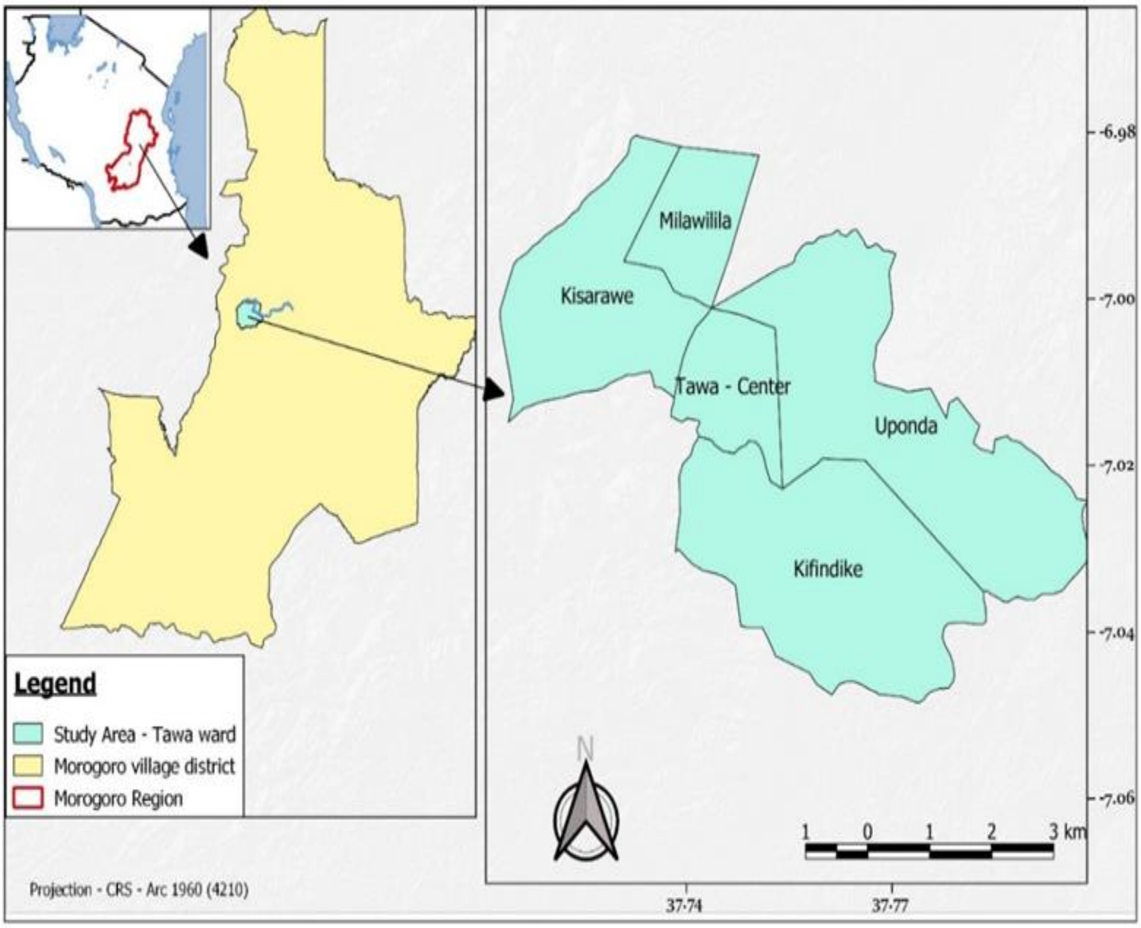

Figure 2.1: Pictorial description of the study area- Tawa Ward

2.3.2 Study population

Clove and black pepper organic farmers from five villages within Tawa Ward formed a study population. The selected villages had existing focus groups that were organized by SAT. Total number of famers who were assessed during this study is shown in Table 2.2.

Table 2.2: Clove and Black pepper farmers involved in this study

Ward	Villages	Name Of A Focus Group	Number of Farmers N=107	
			Clove farmers	Black pepper farmers
TAWA	Uponda	*Hapakazitu*	0	17
	Kisarawe	*Upendo*	9	17
	Milawilila	*Ningendole*	6	10
	Kifindike	*Tupendane*	6	12
	Tawa	*Twaweza*	9	21
		Total farmers	n=30	n=77

2.3.3 Study design

This was a cross- sectional study that involved survey and laboratory analysis.

2.3.4 Assessment of harvesting and post-harvest handling practices

Questionnaires were used to collect qualitative data from 107 farmers in five villages of Tawa. Semi-structured questionnaires were self-administered to farmers who were capable of filling them on their own and assistance was given to farmers who needed as shown in Plate 2.1. The data collected was on regular post-harvest practices performed by farmers. Drying practices and storage facilities were also observed from selected individual farmers according to the prepared checklist.

Plate 2.1: Filing of questionnaires

2.3.5 Laboratory analysis

2.3.5.1 Sample size and sample collection

A total of 34 samples (13 clove samples and 21 black pepper, each weighing between 250 and 500 gm) were randomly collected from interviewed farmers and packed in aseptic sachets and labelled. Samples were categorized according to their availability (Table 2.3). Collected samples were transported to the laboratory under ambient temperature and assessed for moisture content.

Table 2.3: Sampling categories

Spice	Immediately harvested	Dried and stored ≤1 month	Stored for 3 months	Stored for 6 months	Stored for 1 year	Total
Cloves	4	3	3	3	0	**n= 13**
Black Pepper	4	0	11	0	6	**n= 21**
						N=34

2.3.5.2 Moisture content determination

At the laboratory spice samples were ground by using a laboratory blender and sieved across a mesh of 1mm diameter prior to analysis. The moisture content of the spices was determined using an automated moisture measuring instrument (IR35M- 000230V1, Denver Instrument Germany). 2 grams of powdered sample was kept in a moisture meter at temperature of 105°C. The moisture meter uses infrared heat (up to 130°C) to heat a coil that generates heat toward a sample. The heat removes all moisture contained in the sample. The calibrated machine is an automatic moisture analyzer which displays the percentage moisture of the sample that had been calculated by using weight difference between the initial sample and the heated sample.

2.3.6 Data analysis

Descriptive statistics such as frequencies and percentages characterizing the respondents were computed using Statistical package for the social sciences (SPSS) version 20.0. Also, for laboratory data, One-Way ANOVA was done, and results were expressed as means ± standard deviation.

2.4 Results and Discussion

2.4.1 Demographic information of surveyed farmers strata

Clove and black pepper farmers involved in this study were from five villages of Tawa Ward. However, they were considered as a single population as the survey did not reveal any significance difference among farmers from the five villages regarding their post-harvest handling practices of clove and black pepper. Demographic information of the assessed spice farmers (Appendix 2) indicated that clove and black pepper farming in Tawa Ward is dominated by elder male with primary education. More than 80% of all respondents had primary education and 60% among them were male. About 20% of all respondents were aged between 21 and 30 years. Whereas 30% of the remaining percentage were aged between 40- 50 years.

2.4.2 Farming characteristics of clove and black pepper farmers in Tawa

Organic agriculture is a holistic production management system, which seeks to minimize the use of external inputs, avoiding the use of synthetic drugs, fertilizers and pesticides as elaborated in the standard number EAS 456:2007 of East African Standards. Most spices producers in Tanzania are small scale farmers in rural areas who are considered to be growing spices organically by default (ITC, 2014). In the study area, 36.7% and 46.7% of clove and black pepper farmers started to strictly observe organic agriculture about three years ago when an NGO known as Sustainable Agriculture in Tanzania (SAT) visited the area and provided education on organic farming. Others acknowledged having been practicing organic farming even before the awareness of organic farming concept from SAT.

Mixed farming is practiced by more than 96% of clove farmers and more than 97% of black pepper farmers in this study. They mix clove or black pepper trees with both spice and non-spice crop within the same farm space. Mixed cropping system is mentioned to be a common practice among spice farmers in Tanzania (ITC 2014; Reyes *et al.*, 2009). Black pepper and clove are favored by high relatively humid conditions and cannot tolerate excessive heat or dryness. In Tawa, spice farmers depend on seasonal rainfall to water their plants. Almost all farmers declared to control weed by pulling out grasses from the ground and reuse them with other farm wastes for mulching and to improve soil fertility. Only 9.1% and 20% of black pepper and clove farmers mentioned to combine farm wastes with manure and other organic soil improvers to improve soil fertility.

More than 90% of all farmers reported to face pest problem whereas 50% of them said that they take no measure to solve the problem. Others control pest problems by organic means. They use garlic and chili powder to minimize pest infestation on their spice plants. Summary of farmers response on farming characteristics is expressed in percentage at Appendix 3.

2.4.3 Harvesting and post-harvest handling of clove and black pepper in Tawa

Response from interviewed famers on harvesting and post-harvest handling practices are summarized in Appendix 4 and discussed below.

2.4.3.1 Harvesting

Clove and black pepper harvesting require climbing for taller matured trees. During harvesting, matured spices are handpicked from their tress. Handpicking helps farmers to select only matured and less damaged spices. Harvesting of immature crops causes financial losses because immature crop is lighter in weight. Also handpicking minimize bruising of spices that could allow microbial invasion during drying and storage (Karlsson, 2016).

Farmers in Tawa uses a bamboo stick ladder for climbing trees containing matured and ready to harvest spices. A farmer climbs up the bamboo ladder carrying a woven bag across his shoulder for collecting harvested spices as shown in Plate 2.2. Between 5 to 7 kilograms of black pepper and cloves can be harvested from a single well- matured tree during harvesting season. Depending on the farm size and number of matured trees, harvesting of cloves and black paper takes an average of six days per hector.

2.4.3.1.1 Harvesting of black pepper

Pepper fruits mature about 6–8 months after flowering. Under good management, a bush black pepper tree can yield an average of 150–300g of dry pepper per plant/year (Ravindran *et al.,* 2012). In Tawa harvesting of black pepper occurs in two seasons: minor harvesting season (June-August) and major harvesting season on December- January. Locals at Tawa call the minor harvesting season of black pepper *'Mdegeta'* (meaning a little harvest in Luguru language) because the harvesting yields are three times less the major harvesting season.

In this study only 9.1% of black pepper farmers reported harvest more>100kg/hector/ year. This due to mixed cropping farming where black pepper is grown at the same space with other spice and non-spice crops. Intensive intercropping cultivation also known as mixed farming is mentioned to be a common practice by spice farmers in Tanzania. The crops grown in the mixed cropping system observe no definite pattern with regard to the type and number of intercrops involved (Akyoo and Lazaro, 2008).

2.4.3.1.2 Harvesting of cloves

Upon harvesting, cloves are handpicked from the unopened flower buds when are 1.5–2 cm long. The flower buds are borne in clusters of four long calyx, terminating in four spreading sepals, and four unopened petals, which form a small ball in the center. Matured cloves are to be picked before the pinky-green blossoms open. The opened flowers start the cycle of seed generation and are not valued as a spice (Milind and Deepa, 2011). Proper timing is crucial in clove harvesting because it influences grading of clove. Harvesting must also be done without damaging the branches, as it adversely affects the subsequent growth of the tree.

Full bearing of clove tree is achieved by about 20 years and the production continues for 80 years or more. On an average, a clove tree yields 3.5-7.0 kg/year, depending upon the age, size and condition of the tree. Most clove farmers in this study had farm size of less than 2.5 hectors. Only 13.3% of these farmers had harvest of more than 100kg/hector in a year and 30% harvesting only between 10-20 kg per hector per year.

Plate 2.2: Harvesting of clove (a) and Black pepper (b) by using a bamboo stick as a ladder

2.4.3.2 Drying

Drying is done by spreading the harvested spices on mats that are spread directly to the ground. Local mats used during drying are '*bushera*' (made of woven straws), '*kitanga*' (made of dried leaves), '*mkeka*' (made of plastic pipes) and nylon sheaths as elaborated in Plate 2.3. Drying is often conducted under unclean environments, at the open sun in the homestead hence contamination due to dust and livestock become possible. Similar observation is reported in ITC report, (2014).

Cloves and black pepper take an average of 4–5 days to dry depending on the intensity and duration of sunlight (Das and Sharangi, 2018). Semi-dried spices are kept in woven plastic bags and stored inside the house to continue with drying the following day. During drying process, frequently turning of spices is done to ensure even drying.

Spice farmers in Tawa use the following indicators to determine if spices are well dried.

- Color change: Matured and ready to harvest cloves are either green, reddish yellow or red in color (Panco and Montpellier, 2013). Black pepper is either red or green depending on the cultivar and level of maturity. Color changes from green/red to light brown on the first day of drying and continues to change until dark brown for cloves and black for black pepper. All interviewed farmers say color change is the initial indicator of dried spices. They claimed to be experienced enough to relate intensity of color change to level of dryness. But color change itself is not a sufficient indicator of proper drying.
- Pressing by hands and breaking with the teeth: Hand pressing of dried spices to confirm level of dryness has been reported by 63.4% and 44.1% of clove and pepper farmers. Properly dried black pepper is expected to be hard enough to resist tooth penetration. In this study, 26% and 13.3% of interviewed clove and black pepper farmers said they combine this method with color change to decide if the spices are well dried.

- Sound: Dried cloves are expected to produce sound of a dry stick when broken. For black pepper farmers lift a hand full of dried black pepper and pour them down, if they produce a sound similar to the sound of poured dried rice then they are satisfied with the drying condition. Conducted interviews revealed that 20.8% of black pepper farmers rely on this method of deciding level of dryness.

Plate 2.3: Drying of spices in Tawa by using local mats

Good sundried black pepper and cloves should contain no more than 12% moisture (TZS 30 2013; TZS 357 2012). Hence, farmers are required to dry spices from moisture content of above 60% to 12% (Table 2.4). Moisture content of samples collected during this study show that local methods of detecting moisture content could be accurate on black pepper than cloves.

Table 2.4: Moisture content of fresh and dried sample compared to TBS standard

Moisture content (%)	Fresh harvested sample	Dried samples	TBS standard for dried samples (max %)
Clove	60.291±9.176 *(n=4)*	18.87±3.36 *(n=9)*	12
Black pepper	68.146±2.968 *(n=4)*	11.44±1.09 *(n=17)*	12

2.4.3.3 Storage and post-storage handling

Marketing price influence storage time of spices (ITC, 2014). In Tawa middlemen contact the farmers immediately after harvesting to purchase their produces at cheaper price. Storage of spices for longer storage time is common among middlemen than farmers. Middlemen collect and store spices before selling in bulk. Three middlemen who were visited during this study have a selected storeroom for keeping spices throughout the year whereas farmers store spice in their living and bedrooms as shown in Plate 2.4. Storage of spices for three months or less was reported by more than 60% of both clove and black pepper farmers. About 5% of black pepper farmers store their produces for one year or longer.

Stored spices are kept in sisal/plastic woven bags, buckets or metal pots (‘*sufuria*’) depending on their quantities. Living/bedrooms or kitchen roof are common storage places. To prevent moisture re-absorption from the mud floor, spices are kept on a wooden rack base. This was observed in less than 10% of Tawa spice farmers who were involved in thus study.

When interviewed, more than 90% of black pepper farmers mentioned that spices do spoil during storage. Despite the average moisture content of dried and stored black pepper in this study being within acceptable limit of TBS standards, the spiceis reported to be highly susceptible to spoilage than clove (Karsha and Lakshmi, 2010). Farmers make decision to throw away spoiled spices depending on the extent of visible spoilage and contamination. Only 16.9% of farmers who experience spice spoilage, discard them. Others, 27.3% among them admitted mixing spoiled batch with the new unspoiled batch prior to selling, while more than 46% of pepper farmers mention to treat spoiled spices before selling them at cheaper prices. Homebased treatments include rubbing spoiled spices with cooking oil to remove discoloration, blanching, washing with cold water and re-drying in the sun are applied. Spice farmers are mainly concerned on maintaining color of spices by applying the after-spoilage treatments.

Plate 2.3: Storage condition and facilities of spices in Tawa

2.4.4 Quality compromising stages

2.4.4.1 Drying

Spice farmers at Tawa practice open drying. Drying commodities in the sun is the most ancient and traditional method which is still practiced in the tropical zones of our country (Akyoo and Lazaro 2008). This is one of the very cheap methods involving very low investment but increase the chances of spoilage and contamination (Akbulut and Durmus 2009). Once spices are contaminated during drying, other handling practices may also cause additional contamination.

Proper drying is crucial to achieving a high-quality spice product. Ravindran (2012) recommends that moisture content of dried black pepper should be between 9 and 12 for safe storage. In this study the average moisture content on dried and stored spices were 18.87±3.36 for cloves and 11.44±1.09 for black pepper. Farmers used simply visual approaches to decide level of spice dryness. They lack reliable approaches to determine moisture content level. The experience-homebased approaches of moisture determination appeared to be only efficient towards black pepper whose moisture content was within the recommended limits by TBS standard number TZS 30: 2013. For cloves visual color change and breaking of spices to decide level of dryness has shown to be inefficient in the estimation of moisture content. Average moisture content of clove was above the recommended limit of 12% by TBS standards number TZS 357: 2012.

Stability of dried spices increases as the moisture content is reduced. Microbial contamination, physical and chemical changes are both depressed at lower moisture content (Hatamipour *et al.,* 2007).

2.4.4.2 Storage

Compared results between observation checklist and questionnaires exposed that storage environment could be critical in compromising safety and quality of stored spices. This is because of:

- Poor hygiene of storage rooms. When interviewed, more than 89% and 96% of pepper and clove farmers said that they do clean storage places prior to storing spices. However, observation revealed poor hygienic condition as indicated in Plate 2.4.
- Both farmers and middlemen store spices with other things at the same storage space. Farmers store spices in their living and bedrooms while middlemen who were also shopkeepers stored spices in their shops mixed with other items. This may cause cross contamination.
- Inspection of the two large stores used by middlemen to store spices showed that there was roof leakage. The middlemen themselves mentioned that during rainy season they tend to move spices to the angle of the room where the roof is not leaking.

Spices and dried aromatic herbs are susceptible to mold contamination and/or growth if storage conditions are not appropriate. Therefore, they should be stored in an environment with humidity that does not result in the growth of molds. Also, spices and the spice products tend to deteriorate very rapidly. Therefore, the storage facility should contain proper equipment to control temperature, ventilation and relative humidity levels. The place should also be dry and inaccessible from the activities of insects, pests, rodents, birds, livestock, domestic animals and other biotic factors (Das and Sharangi, 2018).

2.4.4.3 Grading

No grading was reported to be done during and immediately after harvesting. However, more than 50% of pepper and clove farmers reported to grade spices based on size, shape and maturity level of the spice seed later, after drying. But they are discouraged to practice grading because grading does not influence pricing. Spice buyers observe three quality traits; proper drying, produce maturity, and absence of physical contaminants. However, even when one or more of these traits is absent, the produce will always be purchased (Akyoo and Lazaro, 2008). Since inspection for these traits is done visually, farmers are lured to conduct intentional adulteration of their produce with various contaminants and other parts of spice trees in an attempt to reduce weight loss. Hence grading become of least importance to these spice farmers.

2.4.4.4 Mixing

Summary of the interviews show that 50% and 48% of clove and pepper farmers said that new batch of spice can be harvested and dried before finishing selling or using the previous batch. More than half farmers among them (23% clove and 27% pepper) admitted mixing the two batches and either continue to store or sell them while mixed together. The rest said that depending on the market price they might sell the two batches separately. Spice mixing is also common among middlemen. Middlemen buy spices in small amount from various farmers. They collect the spices together in a single bag without isolation or means knowing if they were previously contaminated.

2.4.4.5 Post storage treatment

Presence of undesirable changes on spices during storage is a challenge explained by farmers in this study. The changes observed include raise in moisture content (sweating of spices), change in color, breakage of spices into dust, loss of spice original pungency and gain of foul smell. Farmers handle such changes by applying homebased tricks including rubbing with cooking oil, blanching, washing and re-drying. Post storage treatment of spices after spoilage is admitted and explained by more than 46% of black pepper farmers. Spice farmers and middlemen in Tawa are more concerned about maintaining the color of dried spices for marketing. Treatments applied to maintain color after contamination and spoilage indicate that farmers are unaware of the residual toxins that could be left behind by spoilage microorganisms especially fungus.

2.4.4 Farmers knowledge on safety of spices and how customers perception influence quality practices

This study also assessed farmers' knowledge on the causes of spices spoilage and how the customer perception influence quality of the sold spices. Summaries of farmers' responses are indicated at Appendices 5 and 6.

Only 70% and 62% of clove and black pepper farmers admitted knowing organic farming standards despite the training from SAT. All interviewed farmers said that they did not know that there are standard guiding quality and safety of spices. All clove farmers and 87% of pepper farmers mentioned inadequate drying as the major cause of spoilage during storage. And only one farmer knew what mycotoxin ('sumukuvu'in Swahili) was. Unawareness of fungus and their toxins among the interviewed farmers calls for an intervention for spice safety.

More than 93% of spice farmers' customers are middlemen. They approach the farmers earlier and during harvesting season. Middlemen do not ask if the spices are grown organically, hardly ask about the storage time and only complain about quality when there is visible mold growth, hand felt moist and obviously seen dirt. Such trend influence farmers safety practices. This study observed that intervention to improve spice quality at the study area should also involve middlemen.

2.5 Conclusion

Today, increase in production of spices is not the major challenge compared to minimizing their losses. Lack of awareness and improper technology dissemination on pre-harvest approaches, handling, transportation and storage still challenges developing countries (Das and Sharangi, 2018). This study provides further evidence that small scale farmers of spices are struggling on the post-harvest management. Drying is still done on the ground, there is no scientific method of determining moisture content, storage is done under unhygienic environment and grading is influenced by customers who are more concerned of weight than quality. High moisture and humidity level are among the major reasons for spice post-harvest losses and both fungal and bacterial infestation. In this study, mixing of spices and post contamination treatment were among the major quality compromising stages.

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CHAPTER THREE

Manuscript Two

3.0 Fungal Quality and Identification for Organically Grown Clove and Black Pepper in Relation to Their Post-Harvest Handling Practices

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3.1 Abstract

This study assessed the fungal quality in association to post-harvest handling practices applied by organic farmers of cloves and black pepper in Tawa Ward. Farmers who provided samples were interviewed and their drying and storage practices were observed. A total of 34 samples were collected and assessed for moisture content and fungal contamination. Average moisture content was 18.7% (clove) and 12.3% (black pepper). Fungi were isolated by using spread plating method where Potato Dextrose Agar and V8 medias were used. Pure colonies of fungal isolates were further characterized and identified based on macro and microscopic characteristics. All black pepper samples were heavily contaminated by Aspergillus, Rhizopus, Penicillium and Fusarium fungal species at level of 2200 - >30000 cfu/g. Clove sample were contaminated by Aspergillus and Rhizopus species only at lower level of 100-600 cfu/g in 46% of the samples. Longer storage time and mixing spices with previous batches during storage seemed to have higher influence on level of fungal contamination in black pepper along with poor drying and storage practices. The identified species in this study are associated with potential

risk to mycotoxins contamination hence attracts the attention to consumer of cloves and black pepper from Tawa. This study, therefore, recommends improvement in post-harvest handling approaches in order to reduce fungal load and contamination and hence improve the product safety and allow the inherent benefits of cloves of black pepper to reach the ultimate consumers.

Key words: *spices, clove, black pepper, postharvest practices, fungi, Aspergillus spp.*

3.2 Introduction

Clove and black pepper are part of spices whose consumption is usually in small quantities but have wide range of uses in foods and food products (De La Torre Torres *et al.,* 2017). Black pepper is the leading spice commodity worldwide, both by volume and value (Bosland and Votava, 2012) and Clove may be looked upon as a champion of all the antioxidants known till date (Milind and Deepa, 2011).

Both cultivation and trade in spices and herbs have been substantially growing during the last decades (FAOSTAT, 2016). Thus, increasing the importance of quality control measures in spice value chain especially from domestic to export supply chains (Daly, 2015; Kónya *et al.,* 2016). Safety of herbs and spices became an issue of high priority due to presence of both natural or unintentional contamination and the deliberate food adulteration (Schaarschmidt, *et al.*, 2018; Loeffler *et al.,* 2016).

Spices have been reported to contain inherent antimicrobial activity. Mixtures of various spices have even been recommended to replace conventional food preservation additives because of their antibacterial effects (Tajkarimi *et al.*, 2010; Fei *et al.,* 2011; Pina-Pérez *et al.,* 2012; Moore-Neibel *et al.,* 2013). Other health benefits of spices include having antioxidant properties, anti-inflammatory properties and medicinal uses (Peter, 2012b; Charles, 2013; De La Torre Torres *et al.,* 2017).

However, despite being quantitatively minor food ingredients, spices and herbs hold a major potential to contaminate a wide range of products due to the wide-spread use and large-scale distribution (EFSA, 2016; Székács *et al.,* 2017). Spices are usually traded or consumed in the desiccated state. Even at low water activity of desiccated state, spices may provide an environment that allows the survival and persistence of many food-borne pathogens (Gutler and Keller, 2019; Zweifel and Stephan, 2012). Microbiological agents can contaminate spices at numerous vulnerable points within production and supply chains and can pose a serious risk for the consumer (Székács *et al.,* 2017).

Preservative action of herbs and spices is receiving much attention, but studies continue to report, show and prove presence of mycotoxin-producing molds in many spices and herbs (Kabak *et al.,* 2017; Azzoune *et al.,* 2016; Temu 2016, Gutler and Keller, 2019). The mycological quality of some spices on the market, especially of pepper, is quite poor, bearing many genera and species of fungi (Mandeel, 2005; Hashem and Alamri, 2010). Most fungi reported on spices are of the post-harvest and storage type, which develop after harvest under poor control of relative humidity and hygiene during drying and storage (Toma, 2013; Hashem and Alamri, 2010; Hammami, *et al.,* 2014; Casquete, *et al.,* 2017).

In Tanzania, both organic and conventional spices are sold in almost all local markets and are among a food ingredient that are used in almost all households irrespective of their income status. Despite their importance in daily food and food product processes, very little has been done to assess microbial status of spices in comparison to staple crops like maize and cassava (Temu, 2016). This study therefore intended to assess the fungal quality associated with post-harvest handling practices applied by organic farmers of cloves and black pepper in Tawa ward.

3.3 Methodology

3.3.1 Sampling and laboratory sample preparation

A total of 34 dried clove and black pepper samples were directly collected from organic spice farmers located at Tawa Ward. Sampling took place between October 2018 and February 2019. Samples were collected in sterile sachets, transported to the laboratory under ambient temperature then stored in freezing temperature of -18^{o}C while waiting for analysis as per Koburger (1981) and ICMSF (2002). At the laboratory spice samples were ground by using a laboratory blender and sieved across a mesh of 1mm diameter. All equipment that encountered samples (blender and sieve) were sterilized using 70% ethanol prior to and in between each sample. Spice samples in powdered form were kept in sterile sachets and labelled ready for analysis.

3.3.2 Assessment of post-harvest handling practices

Farmers were interviewed and their post-harvest handling practices observed and recorded. Semi structured questionnaires and observation checklist were used to collect both demographic information and post-harvest handling condition of spices by farmers. Samples were collected only from interviewed and inspected farmers.

3.3.3 Moisture content determination

The moisture content of the spices was determined using an automated moisture measuring instrument (IR35M- 000230V1, Denver Instrument Germany). 2 grams of powdered sample was kept in a moisture meter at temperature of 105°C. The moisture meter uses infrared heat (up to 130℃) to heat a coil that generates heat toward a sample. The heat removes all moisture contained in the sample. The calibrated machine is an automatic moisture analyzer which displays the percentage moisture of the sample that had been calculated by using weight difference between the initial sample and the heated sample.

3.3.3 Fungal growth and enumeration

The method used was a modification of the method used by both Hashem and Alamri, (2010) and Pitt and Hocking (2009), in their similar studies. Also, by following guidance from BAM 2005. Fungi were isolated by using agar plate method where Potato Dextrose Agar (potatoes infusion, 200 g/L; dextrose, 20 g/L; agar, 15 g/L; pH = 5.6 ± 0.2) and V8 (clarified V8 juice (100ml) 8.3g/l; L-Asparagine 10 g/l; Yeast extract 2 g/l; Calcium carbonate 2g/l; Glucose 2 g/l; Agar 20g/l Final pH (at 25°C) 5.7±0.2) medias were used. To suppress bacterial growth chloramphenicol (25 mg/l) was added in the medium. Sterile peptone water was used as diluent where appropriate ten-fold serial dilutions (1:10) were prepared starting with 1 g of powdered sample in 9 ml of peptone water, after which 0.1 ml of each diluent was inoculated in the agar. Spread plating of diluent was chosen because fungal colonies grow faster on the surface, gives a more uniform growth and makes colony isolation easier (BAM, 2005). Plates were then incubated (Binder 960285, Germany) upside down at 26°C and undisturbed for 7 days. After incubation, colonies were counted, and the results were expressed in Colony-Forming Units (CFU) /g of samples. Data expressed were the average from the two media used.

3.3.4 Fungal identification

Pure colonies of fungal isolates were obtained by sub-culturing on respective media and were further characterized based on macro and microscopic characteristics. Color and colonies diameter and morphology were used to give initial identification of the fungi genera. The grown fungi were wet mounted using distilled water on a slide, stained with lactophenol-cotton blue for detection (Leck, 1999) and covered with a cover slip. The slide was examined under microscope (Leica GME under×400 magnification) for identification based on their colony morphology and spore characteristics (Ronhede *et al.*, 2005; Rajankar *et al.,* 2007). Texts by Frisvad *et al*. (2005); Pitt, (2000); Mathur and Kongsdal (2003) and Howard (2002) were used in describing the morphology of colony, size, shape, budding, mycelia and fruiting bodies for the identification of Aspergillus, Penicillium, Fusarium and Rhizopus species.

3.3.5 Data analysis

Analysis of single replicate factorial using half normal plot was applied in determine the influence of post-harvest activities to both moisture content and fungal growth of the selected spices. To decide the level of significance, analysis of variance was done.

3.4 Results and Discussion

3.4.1 Characteristics of farmers

Characters of farmers involved in this study are summarized in Appendix 7. Majority are older male with primary education who practices small scale farming of spices. Interview with the farmers reveled that more than 97% are unaware of toxic-producing moulds, mycotoxins and their control.

3.4.2 Fungal quality

The results for moisture content level and fungal contamination in the two spices are summarized in Table 3.1 below;

Table 3.1: Moisture content and percentage of contamination by identified fungi species in studied spice samples

Spice	Average Moisture Content (%, Min-Max)	Level of Contamination	Fungi genera/species	Level of contamination (%)
Clove *n=13*	18.72 (14.32 - 23.31)	46% (100-600 cfu/g)	Rhizopus *spp*	31
			Aspergillus *spp* *(A. flavus)*	15
Black Pepper *n=21*	12.36 (10.13 - 19.58)	100% (2200- >30000 cfu/g)	Aspergillus *spp* (*A.niger, A. flavus, A. paraciticus)*	81 (38,33,10)
			Penicillium *spp*	29
			Rhizopus *spp*	24
			Fusarium *spp* (*F. moniliforme)*	14

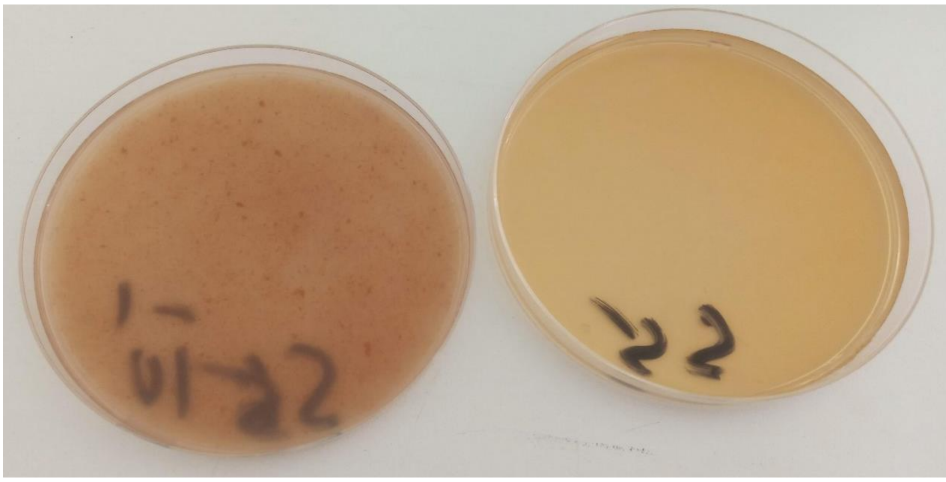
Clove was found to be contaminated by Rhizopus and Aspergillus species at level between 100- 600 cfu/g in 46% of the samples. Higher level of contamination in clove samples was observed to be predominantly of the Rhizopus species. *Rhizopus* are filamentous fungi that can reproduce both sexually and asexually and they are found on a wide variety of organic substrates, including "mature fruits and vegetables" (Kirk *et al.*, 2008). Both *Aspergillus* and *Rhizopus* species are among dominant storage fungi (Bhattacharya and Raha, 2002) and grow fast under humid and moist conditions (Adams *et al.*, 2016). This study detected presence of Rhizopus species in clove samples by using both morphological and microscopic features. Xin, (2012) revealed that clove oil possessed stronger antifungal activities against *Aspergillus flavus* and *Penicillium citrinum*compared to *Rhizopus nigricans*, during both *in vitro* and *in vivo* experiments of observing antifungal activity of clove.

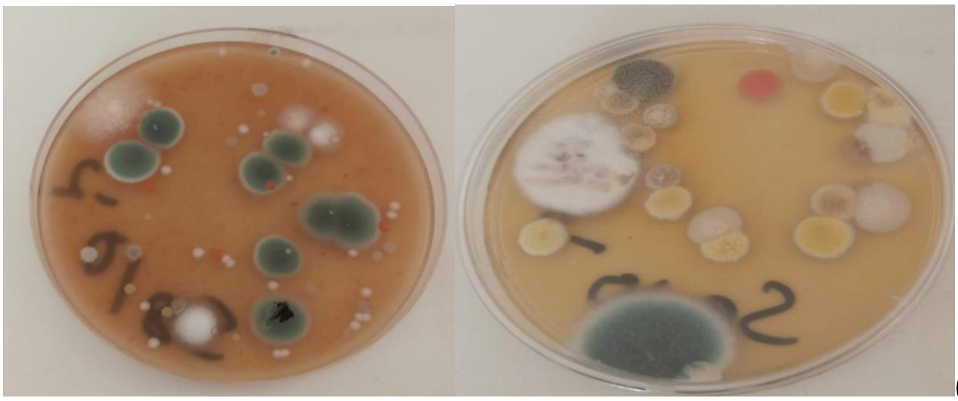
Clove essential oil and active ingredient eugenol is responsible for antimicrobial activity (Pundir *et al.*, 2010). However, antifungal effect may be subjected to bud size and concentration of the clove oil (Omidbeygi *et al.,* 2007; Xin *et al.,* 2012). In a study of using spice oils for the control of co-occurring mycotoxin-producing fungi, whole clove buds were found to have lower antifungal effect compared to grounded clove and extracted clove oil (Juglal *et al.,* 2002).

Hashem and Alamri (2010) and Mandeel *et al.* (2005) reported to detect fungal contamination on clove at level of 163-300 cfu/g by both Aspergillus and Penicillium fungal species. Haruna *et al.* (2016) and William *et al.* (2014) reported presence of both toxin producing molds and aflatoxins at higher than recommended levels in cloves sold at Nigeria and Kenya respectively.

All black pepper samples were contaminated with fungi at level of 2200->30000 cfu/g with four different genera of fungi identified. Aspergillus (both *A. flavus*, *A. parasiticus and A. niger*) was the leading specie followed by Penicillium, Rhizopus and Fusarium species.

Black pepper indicates susceptibility to fungal contamination despite its antibacterial activity (Karsha and Lakshmi, 2010). Most fungi present on pepper are of the post-harvest and storage type, which develop after harvest if relative humidity is not controlled during storage as reported by Aziz *et al.* (1998) and Mandeel (2005). A number of researchers (Freire *et al.,* 2000; Mandeel, 2005; Hashem and Alamri, 2010; Temu, 2016 and Garcia *et al.,* 2018) reported higher levels of fungal contamination by Aspergillus, Fusarium, Rhizopus and Penicillium species in black pepper. Such species were also identified to contaminate black pepper in this study.


(a)


(b)

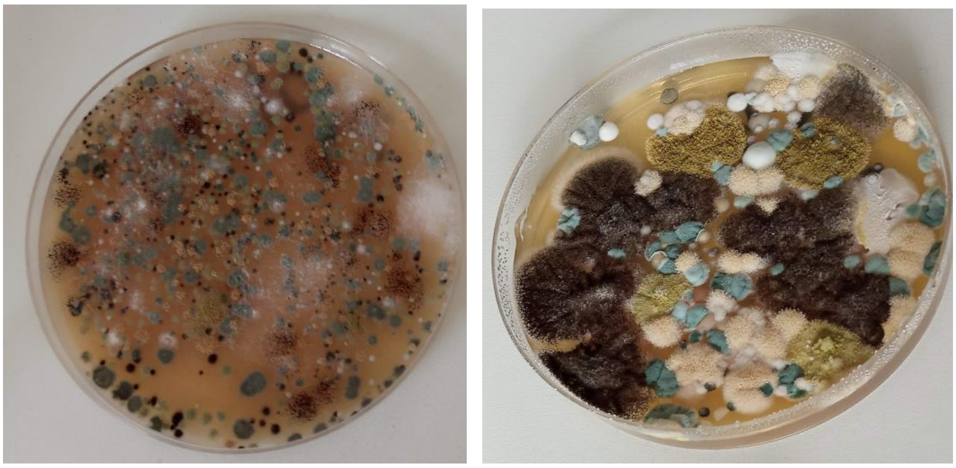
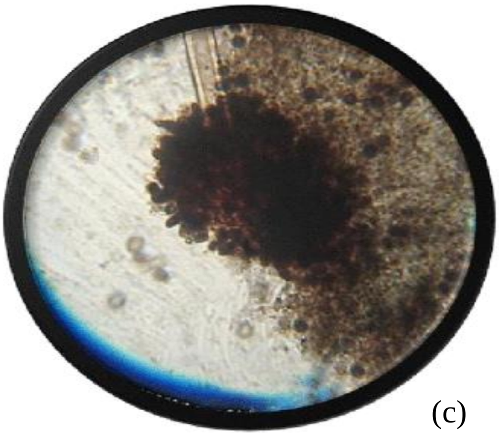

(c)

Plate 3.1: No fungal contamination appeared in 54% of clove samples (a) and low contamination (100- 600 cfu/g) was detected on the remaining 46% of the clove samples (b). Black pepper was heavily contaminated spice with fungi (g 2200->30000 cfu/g) on the both two medias used (c): V8 (left) and PDA (right).

(a)



(c)

(b)

Plate 3.2: Morphological identification of Rhizopus species (a) and microscopic identification (at x 400 magnification) of Aspergillus species; A. niger (b) and A. flavus (c)

3.4.3 Fungal quality and moisture content relate to post harvest handling activities

This study also assessed post-harvest handling of cloves and black pepper in the study area. Results of assessed practices are summarized in Table 3.2.

Table 3.2: Post harvest practiced assessed in this study. Results are expressed in percentage of the total samples in each spice

SPICES	ASSESSED POST HARVEST PRACTISES DRYING METHOD		STORAGE EQUIPMENT			STORAGE TIME				MIXING WITH PREVIOUS BATCH	
	On the Ground	*Raised platform*	*Nylon Sack*	*Sisal sack*	*Plastic bucket/ Metal pot*	*One month*	*Three months*	*Six months*	*One year*	*Yes*	*No*
Clove	84.6	15.4	46.2	23.1	30.8	38.5	38.5	23.0	0.0	0.0	100.0
Black pepper	90.5	9.5	61.9	28.6	9.5	42.9	33.3	0.0	23.8	47.6	52.4

Open air drying is practices by spice farmers in the study area where direct sunlight is used for drying. Spices were either spread on the cover/mat that was in contact to the ground or on the raised platform during drying. Similar method of drying is reported to be practiced by small scale spice farmers in other parts of Africa and Asia (ITC 2014, Das and Sharangi 2018). Nylon and sisal sacks were used for storage of more than 10 kg of spices where smaller amount was just stored in the plastic buckets or metal pots. About 23% of spice farmers claimed to have stored their spices for more than six months. Practice of mixing new batch of spices with previously stored batch was mentioned to be done by more than 46% of black pepper farmers. Such practice could describe the higher level of contamination found in black pepper than clove. Mandeel (2005) point out that both packaging and storage condition influence presence of fungi in cloves. His study observed that samples obtained from gunny bags encounter higher colony counts and contamination frequency than other packing methods. Dried spices can be safely stored up to four years away from sunlight, and under constant hygienic and dry storage conditions (Lawless *et al.*, 2012).

Results from analysis of single replicate showed that all the assessed practices had influence on the moisture content and level of fungi contamination (interaction between mixing of batches to other handling practices has also shown to provide influence on black pepper. However, the analysis of variance showed that both the individual influences and their interactions were not statistically significant at level 0.05% but at lower level of 0.01%.

Moisture content of spices highly depends on proper drying method and prevention of moisture fluctuation during storage. Furthermore, spices collected in tropical areas by simple methods are commonly exposed to many contaminants before being dried enough to prevent microbial growth. Other practices like harvesting, handling and packing, cause additional contamination (Hashem and Alamri, 2010).

Cloves samples were on storage for not more than six months were also not mixed with previous batches. Farmers explained that clove have immediate market than black pepper. Such practices along with inherent clove antifungal effect may explain the absence of fungi contamination in the remaining 53.8% of clove samples. Temu (2016) observed no fungal growth in clove samples obtained from the local market in Tanzania.

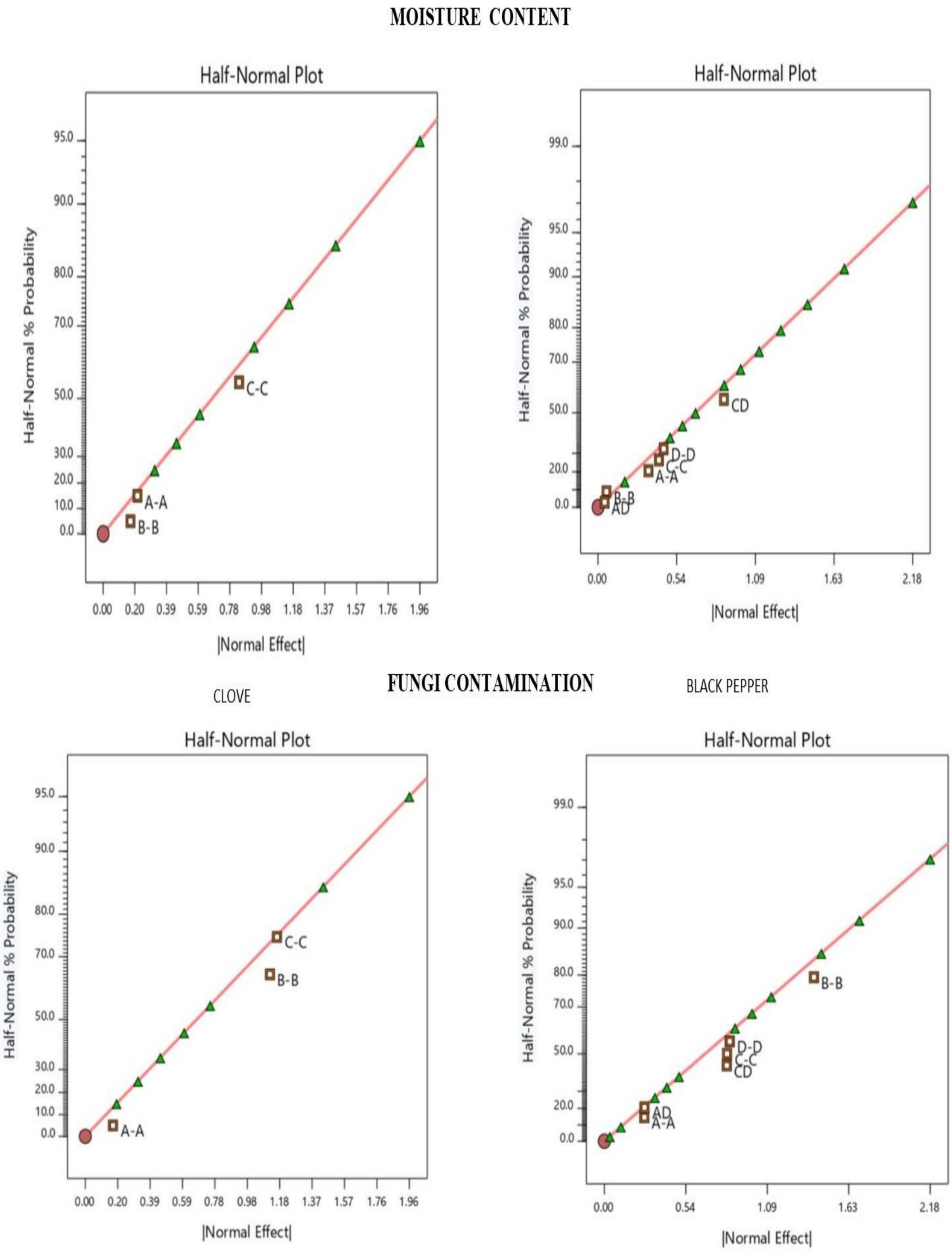


Figure 3.1: Influence of post-harvest practices on moisture content and fungi contamination by using Analysis of single replicate factorial using half normal plot. Where A represent drying method, B- storage equipment C- storage time and D- Mixing new harvest with previous batches

3.5 Conclusion

Both post-harvest practices evaluated in this study have shown influence on the final moisture content and fungal load of black pepper and cloves. Since mixing of spice batches was not a common practice among clove farmers, choices of storage equipment have shown stronger influence on the final quality of cloves as compared to storage equipment and drying methods. In the case of black pepper, the interaction between mixing stored batches with other factors (poor drying and longer storage time) has shown greater influence in the final microbial load and moisture content. *Rhizopus* and *Aspergillus* species were identified from cloves and in addition to them; *Penicillium* and *Fusarium* species in black pepper. Clove antifungal activity was also observed in this study where 46% of samples indicated fungal contamination at lower affinity compared to black pepper despite being obtained from similar conditions.

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CHAPTER FOUR

Manuscript Three

4.0. Lead, Cadmium and Chromium Levels in Organically Grown Clove and Black Pepper from Morogoro Rural District

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4.1 Abstract

Like other organically grown foods, organic spices are expected to be free from synthetic chemicals. However, some research findings show that heavy metal could be found among vegetables that are grown organically. Most food safety studies in Morogoro focused on levels of heavy metals in vegetables grown in urban areas. However, soil, air and human activities can contaminate spices with heavy metals. This study measured levels of Lead, Cadmium and Chromium in organically grown cloves and black pepper by using Atomic Absorption Spectrometry. Level of all the three metals were found to be below permissible levels with maximum average of 0.32±0.22mg/kg (lead), 0.14±0.06mg/kg (cadmium) and 0.33±0.16 mg/kg (chromium). Detection of Lead in 10% of the samples was highly related to poor and unhygienic storage conditions. Despite the detected concentration of Lead, Cadmium and Chromium being below the respective permissible amounts to appear in food, awareness must be imparted due to their bio-accumulative nature. This study therefore recommends further analysis of soil and other spice crops grown in the study area.

Key words: *heavy metals, organic spices, clove, black pepper, lead*

4.2 Introduction

Spice farmers at Morogoro rural district are known to practice organic farming by default (ITC, 2014; Maerere and Van Noort, 2016). IFOAM defines organic farming as a production system that is expected to sustain the health of soils, ecosystems and people by minimizing the use of inputs with adverse effects. Organic farming does not use mineral fertilizers (Muller *et al.,* 2018), and is intended to provide chemical-free food products to its consumers (Saufet *et al.,* 2017). However, studies on bioavailability of heavy metals in organically grown crops established availability of both Lead, Cadmium and Chromium in detectable amounts (Alvarenga *et al.,* 2015; Santos *et al.,* 2009; Weissengruber, 2018, Su *et al.*, 2014; Chen *et al.*, 2013; Żukiewicz-Sobczak *et al.,* 2017; Hattab *et al.,* 2019). Presence and concentration of heavy metal in edible part of plant is influenced by both metallic concentration in the soil and metal-plant affinity (Thanh *et al.,* 2013; Zhang *et al.,* 2013). Green waste compost and other organic matters added to the soil have been mentioned to influence plant uptake of heavy metals. The soluble organic matter acts as a ‘‘cation carrier”, facilitating metal diffusion from soil particles to roots. This is to say if the soil has natural content or is contaminated by heavy metal, use of organic matter in the soil could increase their presence in the plants (Mico *et al.,* 2006; Qing-wei *et al.,* 2007 in Santos *et al.,* 2009; Weissengruber, 2018). Low concentration of soil heavy metals, regardless of being necessary or unnecessary to plants, will not affect the growth of plants in a certain range. But death of plants may occur in too high concentrations beyond plant tolerance thresholds (Qin *et al.,* 1994; Akova 2018).

Lead, Cadmium and Chromium have accumulative behavior and can produce health effects to human (Khan *et al.,* 2015). Despite its role in increasing enzyme activity and play part in carbohydrate metabolism (Al-Fartusie and Mohssan, 2017), Chromium is reported to harm biological systems when consumed in excess (Michalski, 2004; Costa and Klein, 2006; Wise *et al.,* 2016). Lead and Cadmium have no important functions in human body rather play toxic role to living organism, hence are considered as toxic elements (Lokeshwari and Chandrappa2006; Jarup 2003). Prolonged human consumption of foodstuffs contaminated with heavy metals may lead to the disruption of numerous biological and biochemical processes in the human body. Cadmium and Chromium act as carcinogens and Lead is associated with developmental of abnormalities in children (D'Mello, 2003; Wang and Du, 2013; Shadreck and Mugadza, 2013; Flora *et al.,* 2012).

The monitoring of heavy metals in spices is of great importance in protecting the public from the hazards of these metal ions (Seddigi *et al.*, 2016).Many previous studies focused on levels of heavy metals on vegetables grown in urban areas (Saria 2016; Chove *et al.*, 2006; Mwegoha and Kihampa, 2010; Kibasa *et al.*, 2013; Mohamed and Khamis 2012) but then less is known about the levels of heavy metals in both spices and vegetables grown in rural areas of Morogoro region. This study is the first to assess heavy metals in spices grown in rural Morogoro. Studies show that just like other vegetables, spices take up metals from contaminated soil through the crop roots and incorporated them into the edible part of plant tissues or as a deposit on the surface of vegetables (Divrikli *et al.*, 2006). Even though soil represent a major sink for heavy metals to plants (Tom *et al.*, 2014), polluted air due to both agriculture and transportation activities may also become a source of heavy metal contamination to plats (Simon *et al.*, 2011; Tchounwou *et al.*, 2012; Abou-Arab *et al.*, 2015). Several studies highlight that crops growing in soil that containing higher amount of metals have higher chances of metals to be transferred into edible parts of the plant (Tasrina *et al.*, 2015; Antisari *et al.*, 2015; Amour and Mohammed, 2015). Therefore, this study measured levels of Lead, Chromium and Cadmium levels in clove and black pepper grown by employing organic practices at Tawa Ward within Morogoro rural District.

4.3 Materials and Methods

4.3.1 Study area

This study was conducted in two villages; Tawa- center village (07° 00.921' S, 037° 3.878' E) and Kisarawe village (07° 00.755' S, 037° 44.340' E) of Tawa Ward that is within Morogoro rural district involving clove and black pepper organic farmers.

4.3.2 Sample size and sample collection

A total of 30 samples (9 clove samples and 21 black pepper samples) were randomly collected directly from farmers and kept in aseptic sachets. The average size of samples collected ranged between 250 and 500 grams.

4.3.3 Sample preparation

Dry ashing method was applied during sample preparation and digestion. A study that compared sample digestion methods prior to metal analysis, recommended dry ashing as a flexible method (Hseu, 2004). Seggidi *et al.,* 2016 mentioned that drying method described by Aras and Ataman (2007), is among the most commonly used methods for the sample treatment of spices and herbs.

Whole black pepper seeds and clove buds were ground by using a laboratory blender and sieved across a mesh of 1mm diameter. 3g of the ground sample was accurately measured in a pre-heated crucible (at 250°C for 30 min). The crucible with its contents was placed in a muffle furnace and ashed at 450°C for 12 hours. After cooling, 2 ml of 5M HNO3 was added into the ash and evaporated to dryness on a sand bath. The sample was heated again at 400°C for 15 minutes. The ash was digested with 10 ml of 20% (v/v) HCl solution. The solution was swirled then filtered through Whatman No. 42 filter paper.

4.3.4 Heavy metal analysis

A Unicam 919 Atomic Absorption Spectrophotometer (Cambridge, UK) was used to analyze the samples. All stock standard solutions of Lead, Cadmium and Chromium were prepared as per both instruction manual of Unicam 919 and additional guidance from AOAC Official Method 2015.01. A standard air-acetylene flame was used in all determinations. A wavelength of 217 nm, 228.8nm and 357.9nm for Lead, Cadmium and Chromium were set. The lamp current of 12 mA, 10 mA, and 8 mA were used for Chromium, Lead and Cadmium respectively. Both lamps were warmed for 30 minutes prior to analysis with 80% of each lamp current. During analysis standard solutions were run frequently to check the sensitivity of the instrument. Measurements were done against metal standard solutions (Elmer, 2005).

4.3.5 Data analysis

Statistical package for the social sciences (SPSS) version 20.0. was used to compute both descriptive statistics such as frequencies and percentages, and One-Way ANOVA.

4.4 Results and Discussion

4.4.1 Heavy metal concentration

Average of heavy metal concentration in the clove and black pepper samples is indicated in Figure 4.1. There was no significant difference of heavy metal content between cloves and black pepper samples. Abou-Arab *et al.* (2015) showed that there is little variability on the average content of metallic contamination to crops grown in a similar place. Maximum average of heavy metals detected from the samples were 0.32±0.22mg/kg (lead), 0.14±0.06mg/kg (cadmium) and 0.33±0.16 mg/kg (chromium). Cadmium was not detected in any of the clove samples.

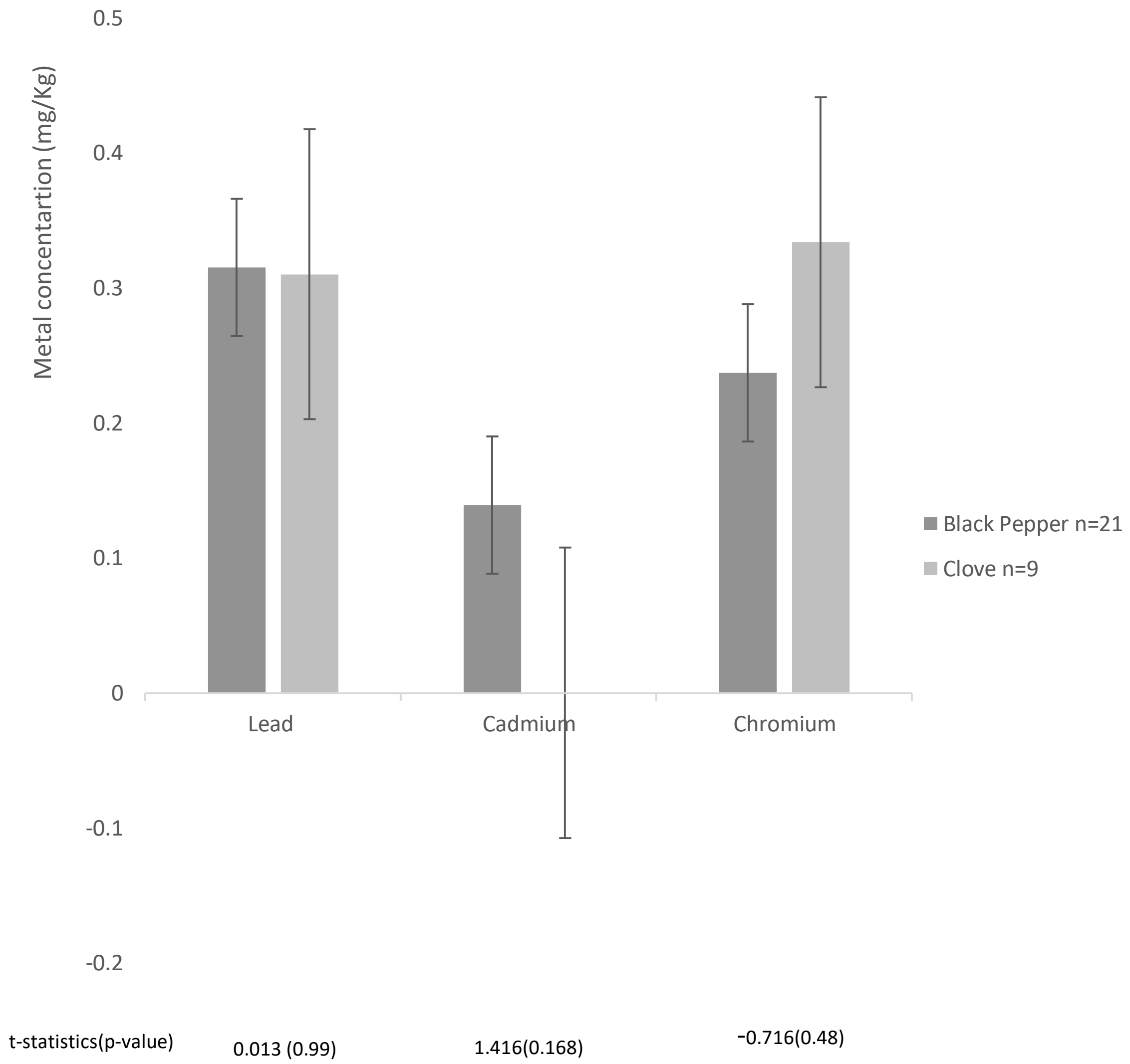

Figure 4.1: Average concentration of heavy metal in spice samples along with their test statistics

The average content of heavy metal into both spices was below the permissible level set by both WHO and TBS standards as shown in Table 4.1 below.

Table 4.1: Comparison of heavy metal concentration in spice samples to permissible levels

	Heavy metals with their permissible* values(mg/kg)					
	Lead (0.3)		Cadmium (0.2)		Chromium (2.3)	
Spices	Mean±S.E	t statistics (p value)	Mean±S.E	t statistics (p value)	Mean±S.E	t statistics (p value)
Cloves	0.31±0.31	0.03 (1.92)	ND	-	0.33±0.16	0.21 (1.68)
Black pepper	0.32±0.22	0.07 (1.88)	0.14±0.06	-0.95 (0.72)	0.24±0.56	-1.12 (0.55)

*The permissible levels as per both WHO and TBS standards; ND= Not detected

Despite the detected concentration of both Lead, Cadmium and Chromium being below the levels stipulated in the standard, awareness creation must be resorted due to their bio- accumulative nature and longer half lifetime (Hezbullah *et al.,* 2016). Foods which are hyper-accumulators of heavy metals should be identified for awareness purposes. Chronic low levels intake of heavy metals such as Lead, Cadmium and Chromium have damaging effects on human beings and their accumulation gives rise to toxic concentrations in the body (Ahmed, 2012). Several studies highlight that crops growing in soil that containing higher amount of metals have higher chances of metals to be transferred into edible parts of the plant (Tasrina *et al.,* 2015; Antisari *et al.,* 2015; Amour and Mohammed, 2015).

4.4.2 Heavy metal distribution among samples

Machine detection level was set to 0.001ppm. However, not all collected samples had detectable amount of heavy metals. Level of detection is elaborated in Table 4.2. Cadmium was not detected in any of clove samples.

Table 4.2: Percentage level of heavy metal detection in the collected samples

	Level of heavy metal detection in spice samples (%)		
	Lead	**Cadmium**	**Chromium**
Clove	11.1	0	55.5
Black pepper	9.5	23.8	57.1

Distribution of Lead in both spice samples is as shown in Figure 4.2 below. Only three samples (two black pepper samples and one clove sample) were detected with Lead content. The concentration average (3.14 mg/kg) of lead from the detected samples was beyond the permissible level of 0.3 mg/kg.

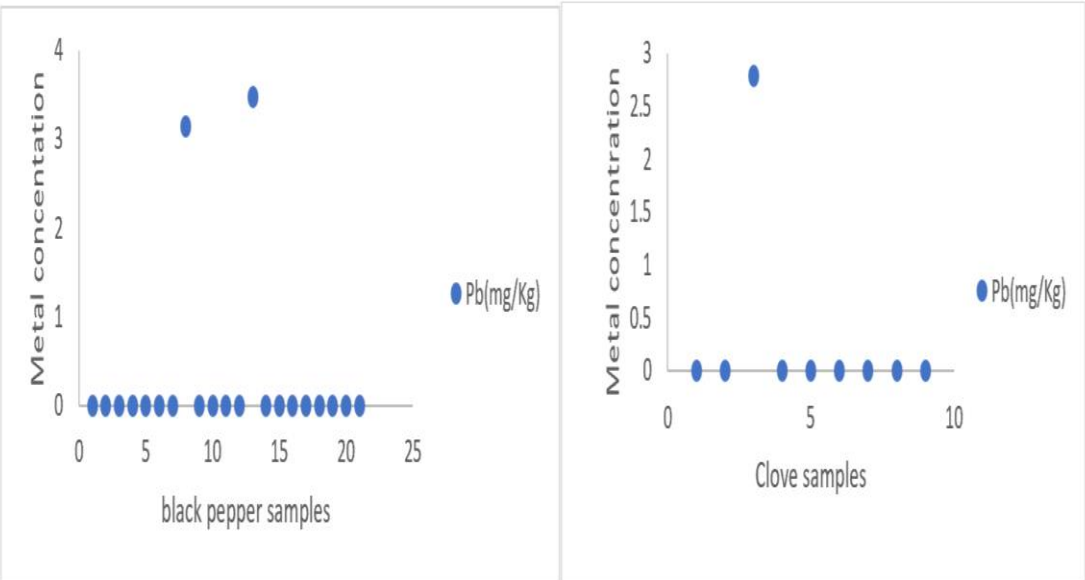

Figure 4.2: Distribution detection of Lead in spice samples

4.4.3 Lead contamination during storage

Tracing the condition of sample collection enable this study to conclude that detection of Lead in 10% of the spice samples was highly related to poor and unhygienic storage practices. Farmers from whom the samples with detectable amount of Lead were collected had poor storage conditions of their spices. Improper arrangement, lack of segregation and presence of charcoal, motorcycle, kerosene and other mechanical instruments in the similar storage facility provided evidence of possible cross contamination. Charcoal, petrol and other pollutants are mentioned to cause Lead contamination in vegetables in urban areas (Abou-Arab *et al.,* 2015). Another study on herbs insist on the significance of harvesting and postharvest safety and hygiene practices and measures in reducing the risk of heavy metal to consumers (Dghaim *et al.,* 2015).

4.5 Conclusion

This work is among the very first studies on heavy metals in the selected area. It saves as a baseline study that can provide foundation and drive to conduct further heavy metal analysis in organic farming areas. It also provides information regarding heavy metal content on the crops that are cultivated by implying organic agriculture practices. Results of this study indicate the possible availability of Cadmium and Chromium in the soil where organic farming is practiced. The detection of Lead in the spice samples was highly related with post-harvest practices. Samples detected with Lead was stored along with other materials that could cause cross contamination. This study also indorses on the significance of hygienic harvesting and postharvest safety in minimizing the risk of heavy metal contamination. Even though, the average amount of all three heavy metals detected were below the permissible limits, this study will raise awareness on the heavy metal situation in the study area.

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CHAPTER FIVE

5.0 Conclusion and Recommendations

5.1 Conclusion

Cloves and black pepper from Tawa are sold within and outside Morogoro region. This study revealed that there is a need to improve and change farmers post-harvest handling practices in order to ensure that safety of such spices along the entire value chain. Drying and storage are still practiced in unhygienic condition and expose the spices to risks of both microbial and chemical contamination. Mixing spice batches during storage has found to accelerate level of fungal infestation. Poor arrangement of storage facility and presence of nonfood materials in storage rooms has resulted in cross contamination of Lead. Both, spice customers ignorance and Farmers lack of knowledge on fungus and mycotoxins in this study was noticed as stimulus to poor postharvest handling practices. Therefore, this study concluded that poor drying and storage activities, mixing of stored spice batches, attempts to treat and retreat already spoiled spices are main causes of poor quality of cloves and black pepper from organic farmers in Tawa.

5.2 Recommendations

This study calls for an intervention on

1. Practical training and Education
    - Training farmers on the proper postharvest management through demonstrations. Farmers continue with improper drying despite more than 87% of interviewed farmers indicating that they knew poor drying is the cause of spice spoilage. That is why this study recommends a practical training that involves demonstrations.

- Knowledge on the causes, effects and control of fungus and mycotoxins is also very crucial in shaping farmers postharvest practices and behaviors. About 47% of farmers in this study attempts to treat spoiled spices in order to retain original color. Little do they know that residues of mycotoxins could be carried on the value chain.

2. Further research

- This study proposes analyzing efficacy of traditional methods used to decide level of dryness in order to decide if they can be improved and promoted to be used by farmers.
- This study recommends further assessment on farmers behaviors and readiness and come up with a model that will enable farmer to adopt to the proper handling practiced.
- Concerning with chemical safety and awareness, this study also recommends analyzing heavy metals profile from soil and other spice crops at the study area.
- Also, further research on identification of pathogenic strains should be done. A quantitative microbial risk assessment evaluation could also be done so as to establish the population exposed to food safety hazard.

3. Moisture meter

- This study recommends use of moisture meter in measuring moisture level during drying and of drying spices. Moisture meter is a fast and yet reliable method that can easily be accomplished by farmers after a simple training. The equipment can be available at handheld level and provide direct readings of the moisture content. Presence of electricity in Tawa Ward, where this study was conducted and existence of farmers in groups makes the suggestion of owning and using moisture meter doable.

4. Applying simple but effective interventions
    - Drying in raised platforms instead of directly in the ground. Simple drying platforms can be built by farmers and be used for spice drying.
    - Cleaning of the storage rooms, proper arrangement and segregation of spices from nonfood materials can easily be done by local farmers once they understand its importance in preventing cross contamination.
    - Avoid moisture re-absorption during storage by not storing spice sacks directly in the floor and prevent roof leakage of the storage room.

In general, the author of this study thinks that spice industry in Tanzania has a potential to growth due to the existence of market and demand around the world. However, since spice industry is dominated by small and middle scale producers, there is still a need for capacity building for the farmers in order to secure sustainable production of cloves and black pepper.

APPENDICES

Appendix 1: Questionnaire

Questionnaire for collecting information on agricultural practices and compliance with required standard from organic spice farmers in Matombo Ward

Dear sir/ madam

My name is Beatha Mkojera from Sokoine University of Agriculture, Morogoro. I am student/ researcher interested in assessing the compliance of organic spices with chemical and microbiological safety standards. I am interviewing farmers on agricultural practices and how such practices can influence the chemical quality of their products. I will ask you some questions but also seek your permission to walk me around your farm to observe your daily activities. Your name will not appear in the final published research. The information I obtain here will be helpful in grading organic spices and will have influence in on the market. You may ask questions at any time throughout our interview. If you have questions about the research after I leave today, you can contact me through my number + 255 713 350 375

Questionnaire No. …………………..

General Information (A)

	Interview	Response
A1	Name of respondent	
A2	Contact (phone number)	
A3	Date of interview	
A4	Age of respondent	21-30 ☐ 31-40 ☐ 41-50 ☐ More than 50 ☐
A5	Sex of respondent	Male ☐ Female ☐
A6	Level of education	No school attendance ☐ Primary school education ☐ Secondary school education ☐ tertiary school education ☐

Assessment of agricultural practices (B)

B1	Have always been practicing the organic farming? If not, when did you shift to organic farming?	Yes ☐ No ☐ …………………………………
B2	How big is your farm?	………………………………
B3	What crops do you grow?	Clove ☐Cinnamon ☐ black pepper ☐ Ginger ☐ turmeric ☐ Other ☐
B4	How do you water your crops? Are you aware of the chemical safety of the water you are using?	Seasonal rain ☐ Irrigation ☐ Yes ☐ No ☐
B5	Do you know the chemical profile of your farm soil? If yes, please provide details.	Yes ☐ No ☐ ……………………………………………
B6	Do your crops get affected by pests? If yes, how do you control them?	Yes ☐ No ☐ Pesticides (industrial made chemicals) ☐ Organically (biochemicals) ☐ Do nothing ☐ Other …………………………………………
B7	How do you control weeds?	Pesticides (industrial made chemicals) ☐ Organically (biochemicals) ☐ Do nothing ☐ Weed removal ☐ Other …………………………………………
B8	Do you ever use agro chemicals? If yes how often? If yes, what did you use them for? If yes, do you remember the names of chemicals you used last season? (please mention)	Yes ☐ No ☐ …………………………………………… …………………………………………… …………………………………………… …………………………………………… ……………………………………………

B9	How do you improve soil fertility?	Organically ☐ • Manure ☐ • Intercropping ☐ • Crop rotation ☐ • Other organic ways ……………… Chemicals ☐ Which ones …………………………..

C. Assessment of drying and storage practise

C1	Where do you dry your spies?	On the ground In kichanja Solar drier Electric drier Other …………………
C2	During drying, do you know how to identify well dried spices? How do you establish that the spices have been well dried?	Yes ☐ No ☐ ……………… ………………..
C3	Do you sort spices? (b) If yes, what aspects do you consider when sorting? ………………………………………	Yes ☐ No ☐
C4	What do you do with the bad sorted spices (rejects)?................................	
C7	How do you store your spies?	In sacks In drums Other ……………………
C8	Do you clean the storage structures before using them?	Yes ☐ No ☐
C9	For how long do you store spices? ………………………………………….	Less than 6 months ☐ For six months ☐ More than 6 months ☐
C10	Do you use any pesticides on spices during storage?	Yes ☐ No ☐ If yes, please mention them …………………………………..
C11	During storage, can you identify spoiled spices?	Yes ☐ No ☐

Assessment on knowledge and awareness

D1	Are you aware of required chemical standard for organic grown spices?	Yes ☐ No ☐
D2	If yes where did you learn about them from?	………………………………
D3	Have you attended any training related to food handling and storage??	Yes ☐ No ☐
D4	Do you know the causes of spoilage in spices? (b) If Yes, please mention the causes you know of …………………………………………	Yes ☐ No ☐
D5	Have you ever heard toxins that may present in crops/food which can be caused by mouldy?	Yes ☐ No ☐
D6	If, provided with the knowledge of guidelines and standards limits for spices, will you be willing to adjust your agriculture practices in order to meet the standards?	Yes ☐ No ☐ Maybe ☐ The decision will depend on ……………………………….

Market assessment

E1	Who are your customers? …………………………………………	
E2	Does your customers know that your spices are organically produced?	Yes ☐ No ☐
E3	Have your customers complained about the quality of your spies? If yes explain ……………………………	Yes ☐ No ☐

Observation checklist

Visual inspection on the dried and stored spices

Criteria	Type of spices	
	Black pepper	Cloves
Overall appearance		
Mould infestation		
Cleanliness		
Moistness (feel by hands)		
Foreign matters		
Others (specify)		
Inspection of the storage place		
General appearance		
Well Organized		
Presence of visible dust		
Spices are kept near non-food materials		
Type of floor (mud/ cement)		
Spice sack contact the floor directly		
Is there roof leakage?		
Is the room ventilated?		

Appendix 2: Demographic information of clove and black pepper farmers in Tawa Ward

	Clove farmers *n=30*	**Black-pepper farmers** *n=77*
	Frequency (Percentage)of respondents	
Village location		
Tawa	9 (30)	21 (27.3)
Kisarawe	9 (30)	17 (22.1)
Kifindike	6 (20)	12 (15.6)
Uponda	0 (0)	17 (22.0)
Milawilila	6 (20)	77 (13.0)
Sex		
Female	7 (23.3)	25 (32.5)
Male	23 (76.7)	52 (67.5)
Education level		
Not attended school	3 (10.0)	6 (7.8)
Primary education	24 (80.0)	68 (88.3)
Secondary education	3 (10.0)	3 (3.9)
Age		
21-30	6 (20.0)	14 (18.2)
31-40	6 (20.0)	23 (29.9)
40-50	10 (33.3)	24 (31.1)
above 50	8 (26.7)	16 (20.8)

Figures in parenthesis indicate percentage of total population (n)

Appendix 3: Farming characteristics of clove and black pepper organic farmers in Tawa Ward

	Clove farmers ***n=30***	**Black-pepper farmers** ***n=77***
	Frequency (Percentage)of respondents	
How long have you been practicing organic farming?	19 (63.3)	41 (53.3)
More than 10 years ago	11 (36.7)	36 (46.7)
3 years ago,		
Farm size (hector)		
0.25-0.4	3 (10)	8 (10.4)
0.5-1.4	16 (53.3)	39 (50.6)
1.5-2.4	7 (23.3)	19 (24.7)
2.5-3.4	1(3.3)	5 (6.5)
3.5-4.4	2 (6.7)	4 (5.2)
>5	13(3.3)	2 (2.6)
Cropping system		
Sole cropping		
Clove/ Black pepper only	1 (3.3)	2 (2.6)
Mixed cropping	13 (43.3)	31 (40.2)
Clove/Black pepper with other spice crops	16 (53.4)	44 (57.2)
Clove/Black pepper with other spice and non-spice crops		
Source of water		
Seasonal rain	30 (100)	77 (100)
Weed problem		
Yes	30(100)	77 (100)
Weed control		
Pull out weed grasses	29(96.7)	77 (100)
Organic weed control	1 (3.3)	
Pest problem	28 (93.3)	71 (92.2)
Yes	2 (6.7)	6 (7.8)
No		
Pest control	13 (43.3)	29 (37.7)
Organic pest control	15 (50.0)	42 (54.5)
Do nothing		
Improving soil fertility	24 (80)	70 (90.9)
Farm wastes	6 (20)	7 (9.1)
Organic soil improvers		

*Figures in parenthesis indicate percentage of total population (n)

Appendix 4: Harvesting and post harvesting practices done by organic farmers in Tawa Ward

	Clove farmers ***n=30***	**Black-pepper farmers** ***n=77***
	Frequency (Percentage)of respondents	
Total harvest kg/ hector/year		
10-20	9 (30)	17 (22.1)
21-40	8 (26.7)	18 (23.4)
41-60	5 (16.7)	15 (19.5)
61-80	2 (6.7)	12 (15.6)
81-100	2 (6.7)	8 (10.4)
>100	4 (13.2)	7 (9.1)
Drying method		
On the ground		
Bushati/kitanga/mkeka/nylon sheath	24 (80)	71 (91.2)
Raised platform		
Kichanja	6 (20)	6 (7.8)
How do you know if spices are well dried?		
Color change only	7 (23.3)	6 (9.1)
Color change and pressing	19(63.4)	34 (44.1)
Color change and sound	0	16(20.8)
Color change and biting with teeth	4 (13.3)	20 (26.0)
Do you grade your spices?		
Yes	15 (50.0)	42 (54.5)
Grading parameters		
Size and shape	12(40.0)	21 (27.3)
Maturity level	3 (10.0)	21 (27.3)
No		
Why not?		
Customers do not care	15 (50)	35 (45.5)
How long do you store your spices after drying?		
≤ 1 month	5 (16.7)	28 (36.4)
3 months	13 (43.3)	25 (32.5)
6months	7 (23.3)	20 (26.0)
≥1 year	5(16.7)	4 (5.1)
Storage equipment		
Sisal sacks	20 (66.6)	29(37.7)
Nylon sacks	8 (26.7)	39 (50.6)
Plastic buckets/ Metal pots	2 (6.7)	9 (11.7)
Do spices spoil during storage?		
Yes	20 (66.7)	70(90.9)
No	10 (33.3)	7 (9.1)
How do you handle spoiled spices?		
Throw away		
Mix with unspoiled batch and sell	10 (33.3)	13 (16.9)
Treat**then sell at cheaper price	3 (10.0)	21 (27.3)
	7(23.3)	36 (46.7)

**Treatment of spoiled spices include rubbing with cooking oil, blanching, rinsing and re-drying

Appendix 5: Farmers' knowledge on safety and organic standards

	Clove farmers *n=30*	**Black-pepper farmers *n=77***
	Frequency (Percentage)of respondents	
Do you know organic standards?		
Yes	21(70.0)	48 (62.3)
No	9 (30.0)	29(37.7)
Do you know quality and safety standards for spices?	0	0
Yes	30(100)	30(100)
No		
Do you know causes of spice spoilage during storage?	30 (100)	67 (87.0)
Yes (Improper drying)	0	10 (13.0)
No		
Do you know mycotoxins?	0	1 (1.3)
Yes	30(100)	76 (98.7)
No		

Appendix 6: Customer perception

	Clove farmers *n=30*	Black-pepper farmers *n=77*
	Frequency (Percentage)of respondents	
Who are your customers?		
Middlemen	28(93.3)	72 (93.5)
Market customer	2 (6.7)	5 (7.5)
Do customers complain about quality of your spices?		
Yes	10 (33.3)	33 (42.9)
Complaints based on		
Visible mold growth	5 (16.7)	8 (10.4)
Improper drying	5 (16.7)	20 (26.0)
General appearance*	0	5 (6.4)
No	20 (66.7)	44 (57.1)
Do customers ask if your spices are grown organically?		
Yes	0	2 (2.6)
No	30 (100)	75 (97.4)
Do customers ask about storage time?		
Yes	7 (23.3)	3 (3.9)
No	23 (76.7)	74 (96.1)

*General appearance involves presence of foreign matters, dirt and stem pieces from a spice tree

Appendix 7: Characteristics of Farmers inspected in manuscript two

	Inspected Farmers *n=34*
Sex	
Female	9 (26.5)
Male	25 (73.5)
Education level	
Not attended school	7 (20.6)
Primary education	22 (64.7)
Secondary education	5 (14.7)
Age	
21-30	7 (20.6)
31-40	4 (11.8)
40-50	11 (33.3)
above 50	12 (35.3)
Farm size (hector)	
0.25-0.1.9	27(79.4)
2.0-4.0	7 (20.6)
Total harvest kg/ hector/year	
< 50	17(50)
51-100	16(47.1)
>100	1(2.9)
Do you know mycotoxins?	
Yes	1 (2.9)
No	33(97.1)