

**ASSESSMENT OF FECAL COLIFORMS IN *Catha edulis*
(KHAT) TWIGS ALONG THE VALUE CHAIN IN IGEMBE
SOUTH SUB-COUNTY, MERU, KENYA**

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**A Research Thesis Submitted in Partial Fulfilment of Requirements for Conferment of
the Degree of Master of Science Sanitation of Meru University of Science and
Technology**

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other Institution.

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DEDICATION

This thesis is dedicated to my parents, my beloved wife, my sons Earl Kipchirchir, Caleb Kipkoech and my daughter Earleen Jeptoo for the moral support they gave me through the study.

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LIST OF ABBREVIATIONS AND ACRONYMS

CFU	:	Colony-forming unit
CLSI	:	Clinical and Laboratory Standards Institute
EMB	:	Ethambutol
IPI	:	International Prognostic Index
MBC/MIC	:	Minimum Bactericidal Concentration / Minimum Inhibitory Concentration
MRCA	:	Most Recent Common Ancestor
NACADA	:	National Authority for the Campaign Against Alcohol and Drug Abuse
SMLs	:	Safe Microbial Limits
SPSS	:	Statistical Package of Social Sciences

DEFINITION OF TERMS

- Customers : Are individuals or businesses that buy khat from vendors for their own use or resale. Customers may not necessarily consume khat themselves but are involved in the purchase for resale to other users. If the study aims to differentiate between direct consumers (users) and those purchasing khat to sell further down the chain, customers may include wholesalers or other bulk buyers
- Different stages : Are the process of khat twigs involves multiple stages, from mature plant selection to consumptions
- Fecal coliforms : are a type of bacteria that are commonly found in the intestines of warm-blooded animals
- Fecal contamination : refers to the presence of fecal matter in water or other environments where it should not be. This can occur due to sewage spills, contaminated stormwater, or other sources
- Handlers : refers to individuals who are responsible for handling, processing, or transporting khat after it has been harvested. This group includes people involved in sorting, packaging, or moving khat to various destinations for sale or distribution. The study population includes 795 handlers
- Indicators : are substances used to show the condition of a solution
- Khat Farmers : individuals who engage in the cultivation of khat (*Catha edulis*) for commercial purposes. This group includes individuals

responsible for planting, maintaining, and harvesting the khat plants. In this study, the term refers to 430 individuals who cultivate khat in Igembe South Sub-County.

Khat : is a stimulant drug made from the twigs and twigs of an evergreen shrub

Microbial contamination : is the presence of microorganisms such as bacteria, viruses, and protozoa in khat twigs

Users : refers to individuals who consume or use khat, typically for its stimulant effects. This includes individuals who purchase and use khat for personal consumption

Value chain : is a series of activities that are performs to deliver khat twigs to customers

Vendors : individuals or businesses that sell khat, either at retail or wholesale levels, within local markets or to other stakeholders. Vendors facilitate the exchange of khat from producers and handlers to the consumers

ABSTRACT

Catha edulis is a plant that is commonly used as a recreational drug in many parts of the world. *Catha edulis* twigs have been found to contain high levels of fecal coliforms, exceeding recommended limits for safe consumption therefore posing high risk of spreading bacterial diseases. The presence of fecal coliforms in these twigs is a concern not only for consumers but also for those involved in their production and transportation. The plant has been grown and consumed in the region around Meru for centuries. Studies have indicated the need for effective measures geared at reducing fecal coliform contamination by *C. edulis*. This study therefore was conducted in Meru County, Igembe South Sub County Kenya between the months of September 2023 and January 2024 to assess fecal coliforms of Khat twigs at different stages of the value chain. This study involved 328 respondents drawn from the 25 locations within the sub- County. It adopted a cross-sectional descriptive study design involving one-time sampling of Khat twigs during farming, handling, vending and consumption. Khat twigs were collected in aerated bags, processed and evaluated for the presence of fecal coliforms thereafter antibiotic sensitivity was tested at Meru University of Science and Technology Health/Biological Sciences Laboratory. Data on factors associated with the fecal coliforms contamination of *C. edulis* were gathered through a structured questionnaire. Data collected was analyzed using statistical package of social sciences (SPSS) version 23.0. Descriptive statistics such as Frequency(n) and Percentage(%)s were used to analyze data. Inferential data were analyzed using T-test and chi square and Tukey HSD to test association. Data findings were presented in tables and graphs besides narrative descriptions. Study findings revealed that handlers had significantly higher fecal coliform contamination with mean difference=0.1162; P=.000 than vendors (mean difference=0.0994; P=.002), customers (mean difference=0.1096; P=.001) and farmers having the lowest with mean difference=0.0169; P=.000. There was a statistically significant association between gender, availability of sanitation facilities, level of education, kind of waste, water source and water storage and fecal coliforms in *Catha edulis* twigs. Ceftriaxone was found to be (100%) susceptible to all bacteria tested. Ampicillin, Amoxyline and Azithromycin showed moderate effectiveness, with around 9-24% of the isolates being resistant while Doxycycline was second most effective drug. The study concluded that fecal coliform contamination levels varied across different stages of the supply chain, with the highest levels found in handlers and the lowest in farmers. Study findings clearly showed that presence of fecal coliforms in khat twigs is significantly associated with the type of sanitation facility, level of education, irrigation practices, water sources, farmers' feelings, waste, water source, and water storage. The study recommends need for improved sanitation practices at all stages of khat value chain. Promoting awareness among farmers, handlers, vendors, and customers about prudent antibiotic use can help combat antibacterial resistance.

CHAPTER ONE: INTRODUCTION

1.0 Introduction

This chapter provides a comprehensive background on *Catha edulis* (khat), highlighting its socio-economic importance, widespread consumption, and health implications. It outlines the problem statement, objectives, research questions, and the justification for conducting the study. This foundational chapter sets the stage for understanding the necessity of evaluating microbial contamination along the khat value chain.

1.1 Background Information

Fecal coliforms are a group of bacteria found in the intestines of mammals, including humans, and are often used as indicators of microbial contamination to depict that the fecal matter is not safely managed from the point of generation, containment, transportation, treatment and disposal or end use (Tominaga, 2019). In addition to causing food spoilage, fecal coliforms can infect animals and humans. The number of coliform bacteria in food is a measure of the hygiene of the cooking process and a potential sign of fecal contamination (Yuan *et al.* 2019). Globally, there has been concern regarding the levels of fecal coliforms found in *C. edulis* twigs, a popular stimulant in many cultures (Sebothoma, 2022).

C. edulis twigs are commonly consumed in Arabian Peninsula. The fecal coliforms are present in these twigs at levels that exceed the recommended limits 1000 CFU/g for safe consumption (Odhaib, Al-Sharafi & Mansour, 2022). The twigs may be contaminated with human or animal feces, which can contain harmful sanitation related germs. According to Debnath (2018), the high levels of fecal coliforms found in *C. edulis* twigs may lead to the spread of bacterial diseases such as typhoid fever, dysentery and diarrheal diseases. The presence of fecal coliforms in *C. edulis* twigs is not only a

concern for individuals who consume these twigs, but also for those who handle them during production and transport (Floom, 2022).

Ingestion of fecal coliforms can result in serious illness, and additionally exposure through contact with contaminated surfaces can also lead to infection. This is a particular concern in regions where proper sanitation is not readily available (McKee & Cruz, 2021). As noted by Othman *et al.* (2019), there is a need for effective measures to reduce fecal coliform contamination in *C. edulis* twigs in order to protect the health of individuals along the production and consumption chain.

There are several factors that can contribute to fecal coliform contamination in *C. edulis* twigs. One of these is the use of contaminated water during the cultivation process (Zacharia *et al.*, 2019). The use of untreated or improperly treated water in irrigating crops can lead to high levels of fecal coliforms in the final product. Additionally, poor hygiene practices by individuals that handle the twigs can also contribute to contamination (Amoah, Kumari & Bux, 2022). This is a particular concern in areas where there is little education or awareness about the importance of sanitation such as proper use of toilets. Efforts to reduce fecal coliform contamination in *C. edulis* twigs must focus on a multi-faceted approach. This includes better regulation of the use of water resources during cultivation, improved hygiene practices, education and awareness campaigns, and regular testing and monitoring of the twigs (Hanna, 2019). The use of good agricultural practices can significantly reduce the levels of fecal coliforms in *C. edulis* twigs (Debnath, 2018). It is essential for governments and other stakeholders to work together to implement effective measures to ensure the safety of this popular stimulant.

Regionally, *C. edulis* is commonly chewed in many African countries due to its numerous pharmacological effects. However, the widespread consumption of *C. edulis*

twigs poses a significant health risk as the plant may be contaminated with pathogenic microorganisms, including fecal coliforms, which are indicators of microbial contamination (Al-Maweri *et al.*, 2018). Therefore, understanding the relationship between fecal coliforms and microbial contamination of *C. edulis* twigs is crucial in ensuring public health safety.

The prevalence of fecal coliforms in *C. edulis* twigs in Africa varies depending on the region where the plant is grown (Floom, 2022). *C. edulis* twigs may be contaminated with fecal coliforms exceeding Safe Microbial Limits (SMLs). For instance, in Ethiopia *C. edulis* twigs sampled from an irrigation canal had a fecal coliform concentration of 1600 CFU/g, exceeding the SML for vegetables (1000 CFU/g) (Ayano, Ayalew, Bedaso & Duko, 2022). Further, in Africa, several factors contribute to the prevalence of fecal coliforms in *C. edulis* twigs. Hygienic practices during harvest and transportation may also contribute to the prevalence of fecal coliforms. The location of the plant, the soil pH, and the water source are other factors that may significantly affect the growth and presence of fecal coliforms in *C. edulis* twigs.

In Tanzania, khat harvested from farms near informal settlements has been found to be highly contaminated with fecal coliforms. High levels of other bacteria such as *Salmonella* Spp. and *E. coli*, which pose a significant public health challenge to khat users in the country (Suleiman *et al.*, 2018). Furthermore, the level of fecal coliforms in khat is directly proportional to the distance between the farms and wastewater treatment facilities. Studies have established that khat farms located close to wastewater treatment facilities are likely to be highly contaminated with fecal coliforms (Horváth *et al.*, 2022; Ademe *et al.*, 2020). Hence, it is essential for farmers to ensure that the wastewater disposal system meets the required standards to minimize the risk of contamination. Apart from contamination from wastewater treatment plants, khat can get contaminated

during harvesting and transportation. In Ethiopia, studies have identified human activities such as handling khat with unclean hands as a significant contributor to bacterial contamination (Gebreyesus *et al.*, 2017; Ademe *et al.*, 2020). Similarly, khat is highly susceptible to contamination from soil and water during harvest and transportation, which could expose consumers to pathogens such as *E. coli*.

The current Percentage(%) of Kenyans using khat varies by gender and geographic region, but it is 3.9% overall (Ndubi *et al.*, 2023). The practice of chewing khat is being condemned by non-users in both rural and urban areas, but its use is on the rise, especially among young people. The practice of chewing khat for recreational purposes is on the rise in metropolitan areas. Much like cigarette use, miraa is mostly associated with men. According to a survey by NACADA, 35.8% of men in the North Eastern region reported using Miraa. The Coast region came second, with 12.8%. In Western Kenya and Nyanza, Miraa is barely used (Japheth, 2020). The majority of the miraa originates in the eastern region. An intriguing fact to keep in mind is that the North Eastern region had the highest proportion of female Miraa users at 7.6% (NACADA, 2012).

Microbial contamination is a common phenomenon in khat, with various bacteria and fungi present at different levels in Kenya. According to a study by the National Institute for Medical Research in 2020, of the 30 samples tested in Nairobi, 50% had microbial contamination (Nyongesa *et al.*, 2020). The most prevalent microorganisms were Enterobacteriaceae, Bacillus spp, Aspergillus spp, and Penicillium spp. In Meru County, similar study conducted in different market found contamination levels ranging from 25% to 60% (Kiraithe *et al.*, 2018). These findings show that microbial contamination is a prevalent problem in khat commerce.

The sources of microbial contamination in khat are varied and can occur at different stages of the supply chain. Fungal contamination can occur during growth, harvest, storage, transportation or drying of the twigs. The ubiquitous nature of microorganisms means that they can also thrive in unsanitary conditions and contaminated water used during cultivation or preparation. The handling of khat by different people can also contribute to contamination. Another study have found that the hygienic practices for khat handlers were poor, and this increases the potential for microbial contamination (Nyongesa *et al.*, 2020).

The consumption of contaminated khat poses a health risks to consumers. The health implications of microbial contamination can range from mild to severe symptoms depending on the virulence of the microorganisms present. The symptoms include vomiting, diarrhea, stomach pain, fever, and respiratory infections (Kiraithe *et al.*, 2018). Some microbial species, such as *Aspergillus Spp.* and *Penicillium Spp.*, produce toxins that can cause liver damage or cancer. The risk of exposure to harmful microorganisms is higher for regular khat users, who may develop chronic contamination-related diseases. These individuals rarely receive medical examinations, even though Kenya's health regulations require all food handlers to undergo regular medical examinations to prevent the potential spread of disease-causing agents (Kamau *et al.*, 2012). This study was conceived in light of this context.

1.2 Statement of the Problem

The prevalence of fecal coliform in Khat twigs in Meru has increased significantly over the past few years (Oyugi *et al.*, 2024). In 2020, for example, researchers reported that around 40% of the Khat samples they tested were contaminated with fecal coliform (Ripanda *et al.*, 2021). By 2019, this figure had risen to 60%, which is a significant increase (Drechsel *et al.*, 2022). The rise in contamination is particularly worrisome

because fecal coliform is a type of bacteria that originates from human feces, and its presence in Khat twigs suggests that the plant is being grown and harvested in unsanitary conditions where sanitation situation is poor or none at all. The current situation in the country calls for understanding of faecal contaminations in various food stuff and edible substances.

The increase in fecal coliform contamination of Khat twigs in Meru has serious health implications for the consumers of the substance. In severe cases, the infection can even lead to kidney failure and death, particularly in individuals with weakened immune systems such as infants, pregnant women, and the elderly.

The consumption of contaminated Khat twigs, therefore, poses a significant risk to the health and wellbeing of the individuals consuming it.

However, it is important to note that the presence of fecal coliforms does not necessarily mean that harmful pathogens are present, as some fecal coliforms are harmless to humans. Nevertheless, the presence of fecal coliforms in khat twigs at different stages of the value chain can indicate that the product is being handled in places where there are poor sanitation and as a result the potential for introduction of harmful pathogens, which can pose a risk to human health once these twigs are consumed.

Studying fecal coliforms as indicators of microbial contamination of khat twigs at different stages of the value chain can help identify potential sources of contamination and develop strategies to prevent contamination. Therefore, this study sought to assess fecal coliform as indicators of microbial contamination of *Catha edulis* (Khat) twigs at different stages of value chain in Igembe South Sub-County, Meru, Kenya.

1.3 Research Questions

- i. What is the status of fecal contamination of *C. edulis* twigs at different stages of value chain in Igembe South Sub-County, Meru, Kenya.

- ii. What factors are associated with the fecal coliforms contamination of *C. edulis* twigs at different stages of value chain in Igembe South Sub-County, Meru, Kenya?
- iii. Do bacterial isolates from khat twigs respond to commonly used antimicrobial agents?

1.4 Objectives of the Study

1.4.1 General objective

To assess fecal coliforms as indicators of microbial contamination of *Catha edulis* (Khat) twigs at different stages of value chain in Igembe South Sub-County, Meru, Kenya.

1.4.2 Specific objectives

- i. To assess the rate of fecal coliforms contamination of *Catha edulis* (Khat) twigs at different stages of value chain in Igembe South Sub-County, Meru, Kenya.
- ii. To determine the factors associated with the fecal coliforms contamination of *Catha edulis* (Khat) twigs at different stages of value chain in Igembe South Sub-County, Meru, Kenya
- iii. To determine the effectiveness of commonly used antimicrobial drugs on bacterial isolates from khat twigs in Igembe South Subcounty, Meru, Kenya

1.5 Jusfication and Significance of the Study

The study on the assessment of fecal coliforms as indicators of microbial contamination of *C. edulis* (khat) twigs at different stages of the value chain in Igembe South Sub-County Meru, Kenya is significant for several reasons. Firstly, fecal coliforms are frequently employed as indicators of potential sewage contamination due to their prevalence in human and animal excrement. The detection of fecal coliforms in khat twigs may suggest the existence of pathogenic bacteria, viruses, and protozoans that are also found in the digestive systems of humans and animals. Hence, the research could aid

in the detection of potential health hazards linked to the ingestion of khat twigs contaminated with fecal matter.

Secondly, the study helps to identify potential sources of contamination of khat twigs along the value chain. The researcher collected samples of khat twigs at different stages of the value chain, including harvesting, transportation, and retail, and analyze them for fecal coliforms. By identifying the stages of the value chain where contamination is most likely to occur, the study could help to develop strategies to reduce contamination and improve the safety of khat twigs for consumption.

Thirdly, the study could contribute to the development of guidelines and regulations for the production and sale of khat twigs. The study could provide evidence to support the development of regulations that require khat twigs to be tested for fecal coliforms before they are sold to consumers.

Lastly, the findings could benefit future researchers by providing data on which further studies regarding fecal coliforms as indicators of microbial contamination of *C. edulis* twigs at different stages of value chain.

1.6 Limitation of the Study

The study on the assessment of fecal coliforms as indicators of microbial contamination of *C. edulis* twigs at different stages of the value chain may face some limitations. One limitation could be the sample size. If the sample size is too small, the results may not be representative of the entire population. To mitigate this, the researchers increased the sample size to ensure that the results are statistically significant.

Another limitation could be non responsive/ cooperative respondents leading to small sample size and this could lead to non representation of the whole population.

1.7 Delimitation of the Study

Delimitation is the process of defining the scope of a study. In the study on the assessment of fecal coliforms as indicators of microbial contamination of *Catha edulis* (khat) twigs at different stages of the value chain, delimitation would involve defining the specific aspects of the study that investigated. The study focused on the presence of fecal coliforms as indicators of microbial contamination of khat twigs at different stages of the value chain. The study did not investigate other types of microbial contamination or other types of indicators of fecal contamination.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

This chapter reviews relevant literature on the microbiological safety of fresh produce, with an emphasis on leafy crops and khat. The chapter also discusses previous studies on food safety, value chain analysis, and post-harvest handling practices. By synthesizing existing knowledge, this chapter builds the theoretical and empirical basis for the study and identifies research gaps the present work aims to fill.

2.1 *Catha edulis* (miraa)- Classification

Khat, or *C. edulis*, is a plant species in the Celastraceae family (Mworia *et al.*, 2008). It is a member of the Plantae kingdom and the Magnoliopsida class. Originating in Ethiopia, *C. edulis* eventually made its way to Somalia, Yemen, Kenya, Uganda, Congo, Tanzania, Zambia, Zimbabwe, Afghanistan, and Madagascar (Nthiga, 2022). According to Al-Juhaishi, Al-Kindi, and Gehani (2012), the majority of the 5 to 10 million people who chew *C. edulis* are located in the Arabian Peninsula and the Horn of Africa. Both Ethiopia and Kenya produce and consume *C. edulis* (Tembrock *et al.*, 2017). In its native habitats on the Arabian Peninsula and the Horn of Africa, the khat plant is known by a number of descriptive names, including Kafta, Jimaa, Miraa, Somali Tea, Abyssinian Tea, and Arabian Tea (Nyongesa and Onyango, 2010). Usually reaching a height of 1–5 meters (3–3 inches – 16 feet 5 inches), khat is a slowly expanding shrub or tree.

2.2 *Catha edulis* Uses

Worldwide, khat is used daily by around 10 million people. Communities in the Arabian Peninsula and the Horn of Africa are the primary growers of khat because the practice dates back thousands of years as a social norm there (Bekerleg, 2008). Women in Yemen chew khat less formally and less often than men do, as is traditional in the country.

About 70–80% of Yemenis aged 16–50 chew khat occasionally, according to researcher (Al-Maweri *et al.*, 2024). A staggering 14.6 million Yemenis smoke khat every hour of every day, according to estimates. Additionally, studies have shown that households spend around 17% of their income on khat (Hussein *et al.*, 2023).

2.3 Rate of Fecal Coliforms Contamination of *Catha Edulis* (Khat) Twigs at Different Stages of Value Chain

The use of Khat twigs has been associated with various health implications, including the contamination of fecal coliforms. The rate of fecal coliforms contamination in Khat twigs at different stages of value chain is a significant concern. Fecal coliforms are commonly used as indicators of possible fecal contamination because they are commonly found in human and animal feces (Weis *et al.*, 2018). Although they are generally not harmful themselves, they indicate the possible presence of pathogenic bacteria, viruses, and protozoans that also may be present. Fecal coliforms are considered to be more directly associated with fecal contamination from warm-blooded vertebrates than are other members of the coliforms (Hamed, 2020). *C. edulis* twigs can be contaminated with human parasites. A study conducted in Nairobi City County, Kenya by Nthiga *et al.*, (2019) found that the main contaminants of *C. edulis* twigs were human parasites, and the presence of parasites was statistically associated with the area/site and the type of waste. Another study evaluated the toxicological effect of varying levels of *C. edulis* twigs on animals and found that the twigs caused signs of liver damage (Githua *et al.*, 2022).

The first stage of the Khat value chain is harvesting. During this stage, factors such as hygiene and sanitation significantly determine the level of fecal coliforms contamination. Firstly, the hygiene of the farmers is an essential determinant of fecal coliforms contamination at this stage (Tuffa, 2019). If Khat farmers do not wash their hands after

using the toilet, fecal coliforms can be transmitted to the harvested Khat twigs. Secondly, the use of contaminated manure can also increase the rate of fecal coliforms in Khat twigs.

The third stage of the Khat value chain is transportation. During this stage, the handling of Khat twigs can influence the level of fecal coliforms contamination (Al-Barakani, 2021). Firstly, the mode of transportation can contribute to the rate of contamination. For instance, if the transportation containers are not cleaned properly, fecal coliforms stuck to the surface, can further contaminate the Khat twigs. Secondly, the duration of transportation can also affect the rate of contamination. The longer the Khat twigs stay in transit, the higher the possibility of contamination (Alemu & Tegegne, 2022).

The fourth stage of the Khat value chain is selling. During this stage, the handling and storage of Khat twigs can influence the level of fecal coliforms contamination (Orr, Donovan & Stoian, 2018). Firstly, the hygiene of the sellers is an essential determinant of fecal coliforms contamination at this stage. If Khat sellers do not wash their hands, fecal coliforms can easily be transmitted to the Khat twigs. The storage of Khat twigs can also increase the rate of fecal coliforms in Khat twigs. If Khat twigs are stored in unclean containers, it can lead to the proliferation of fecal coliforms.

The final stage of the Khat value chain is consumption. During this stage, the handling and preparation of Khat twigs can influence the level of fecal coliforms contamination (Ripanda *et al.*, 2021). Firstly, the hygiene of the consumers is an essential determinant of fecal coliforms contamination at this stage. If Khat consumers do not wash their hands, fecal coliforms can easily be transmitted to their bodies. The food preparation equipment can also increase the rate of fecal coliforms in Khat twigs. If the knives and chopping boards used for Khat twigs are not clean, they can facilitate the spread of fecal coliforms.

2.4 Antimicrobial Susceptibility Patterns of Bacterial Isolates From Khat Twigs

Ankomah *et al.* (2021) suggested that khat extracts had potential applications as antimicrobial agents, particularly against oral bacteria. The extracts have been shown to possess selective antibacterial activity against oral microorganisms, including periodontal disease-associated bacteria such as *Porphyromonas gingivalis* and *Tannerella forsythensis* (Othman, Sleiman & Abdel-Massih, 2019). In addition, there is preliminary evidence for the presence of an antibiotic resistance-modifying component in khat extracts (Palai, 2024).

The response of bacterial isolates from khat twigs response to commonly used antimicrobial agents is a crucial step in determining the effectiveness of these agents in treating bacterial infections. Previous research has shown that complex herbal mixtures containing several plant-based ingredients have been described as formulas for traditional Ayurveda and Chinese herbal medicine (van Vuuren & Viljoen, 2011). This suggests that natural remedies may offer alternative options for treating bacterial infections. However, the development of resistance to antimicrobial agents among bacteria and their side-effects cannot be ignored (Saquib *et al.*, 2021). As such, ethnopharmacology has gained attention as an area of study due to its focus on developing new treatments using natural sources while minimizing negative side effects. The fungal extract demonstrated a bactericidal effect on MRSA with an MBC/MIC ratio equaling one (Muazzam & Darah, 2021). This finding presents a promising possibility for future research into potential natural remedies or alternatives to conventional antibiotics. Furthermore, it was found that some of these isolates were resistant to certain types of antibiotics. However, other antimicrobials like ciprofloxacin showed potent activity against most strains tested. It is essential to consider not only the efficacy but

also potential toxicity associated with using various medications when devising treatment plans for patients suffering from bacterial infections.

Vaou *et al.* (2021) conducted a study on bacterial isolates found on khat twigs. The results of the study revealed that different antimicrobial agents showed varying levels of susceptibility against the bacteria found in khat twigs. This finding suggests that not all antibiotics may be effective against these types of bacteria, highlighting the importance of proper diagnosis and treatment when dealing with such infections. Moreover, this study sheds light on the potential risks associated with consuming khat twigs. While traditionally used for its stimulating effects, it appears that there may be hidden dangers lurking within its consumption - particularly when considering potential bacterial infections. These findings also underscore the need for continued research into antibiotic resistance and development to help combat increasingly virulent strains of bacteria.

Al-Haddad *et al.* (2021) suggested that some commonly used antimicrobial agents may not be effective against bacterial isolates from khat twigs. This finding is particularly concerning given the increasing popularity of khat as a natural stimulant and its potential for spreading drug-resistant bacteria. The study's results suggest that more research is needed to better understand how these bacteria develop resistance to certain antimicrobials and identify alternative treatments that can effectively target them. Moreover, this discovery calls into question the effectiveness of current treatment protocols for patients exposed to khat twigs or other contaminated substances. Health professionals should remain vigilant when treating infections linked to exposure to such substances and consider alternative strategies where necessary. Furthermore, this highlights the need for continued research on novel antibiotics with different mechanisms of action than those currently available in clinical practice.

Saquib *et al.* (2021) investigated the synergistic antibacterial effect of combining ethanolic extracts from *Punica granatum*, *Commiphora molmol*, and *Azadirachta indica* with antibiotics to treat periodontopathic bacteria. The results were promising as all plant extracts exhibited inhibitory effects on bacterial growth. Furthermore, when used in combination with antibiotics, a synergistic antibacterial activity was observed indicating that herbal extracts could provide an effective alternative treatment for bacterial infections. This is particularly relevant for khat twigs which have been found to harbor high levels of bacteria including potential pathogens such as *Salmonella* spp., *Klebsiella pneumoniae* and *Staphylococcus aureus* (Zenebe, Molla, Beza & Mekonnen, 2021). These bacteria can cause serious health problems such as gastrointestinal illnesses and skin infections making it imperative to find safe yet effective treatments. Traditional antibiotic therapy has limitations due to antibiotic resistance leading researchers to explore alternatives such as herbal medicine. Therefore, the use of natural products like plant extracts represents a viable option that warrants further exploration for treating bacterial infections associated with khat twigs.

2.5 Factors Associated With the Fecal Coliforms Contamination of *C. Edulis* Twigs at Different Stages of Value Chain

There is limited information available on the factors associated with fecal coliform contamination of *C. edulis* twigs at different stages of the value chain in Kenya. However, it is known that khat twigs are commonly chewed, brewed into tea, sprinkled on food, or smoked (Nthiga, Mbugua & Anjiri, 2019). Nthiga (2022) found that the khat twigs were contaminated with various parasites, including *Entamoeba histolytica*, *Giardia lamblia*, and *Ascaris lumbricoides*. While this study did not specifically investigate fecal coliform contamination, it suggests that khat twigs may be contaminated with various pathogens.

Fecal coliforms are bacteria that are commonly used as indicators of fecal contamination in food and water (Salamandane, Vila-Boa, Malfeito-Ferreira and Brito, 2021). They are associated with fecal contamination from warm-blooded vertebrates. Pathogenic strains of *Escherichia coli* (*E. coli*) are transferred to seafood through sewage pollution of the coastal environment or by contamination after harvest (Khan & Gupta, 2020). *E. coli* food infection causes abdominal cramping, water or bloody diarrhea, fever, nausea, and vomiting. While this information is not specific to khat twigs, it suggests that fecal coliform contamination may be a concern for any food that is at risk of fecal contamination. It is important to note that khat twigs have stimulant effects and are similar to strong coffee in their effects on the central nervous, cardiovascular, respiratory, endocrine, gastrointestinal, and genitourinary systems.

2.5.1 Pre- and post-harvest elements

The length of time that *C. edulis* twigs are stored can have a significant impact on their level of fecal coliforms contamination. The twigs are stored for longer periods, the level of contamination increases. This is because as the twigs age, they become more susceptible to bacterial growth, and the number of fecal coliforms present can increase drastically (Turner, Luo & Buchanan, 2020). Additionally, if the twigs are not stored properly, the risk of bacterial growth and contamination can increase even further. To mitigate this risk, it is recommended that the twigs be consumed as soon as possible after harvesting, and that any leftovers be stored in a cool, dry place for no longer than one day (Dunn, 2022).

In addition to storage time, storage temperature is another key factor that can impact the level of fecal coliforms contamination on *C. edulis* twigs. The optimal temperature for storing the twigs is around 5 °C (Mir *et al.*, 2018). At this temperature, bacterial growth is slowed, and the level of contamination is reduced. However, if the temperature is too

high, the twigs can become a breeding ground for bacteria, and the level of contamination can increase. Similarly, if the twigs are stored in cold temperature, they can become damaged, which can also increase the risk of bacterial growth and contamination.

The circumstances where *C. edulis* twigs are harvested can also have an impact on their level of fecal coliforms contamination. Twigs harvested from areas with poor sanitation and hygiene practices are more likely to be contaminated with fecal coliforms. Additionally, if the twigs are harvested from areas where animal waste is frequently present, the likelihood of contamination is also increased (Kayombo & Mayo, 2018). Therefore, it is important to ensure that the twigs are harvested from areas with good sanitation practices, and that they are washed thoroughly before consumption.

The processing techniques used on *C. edulis* twigs can also impact their level of fecal coliforms contamination. Twigs that are washed thoroughly before consumption have a lower likelihood of being contaminated with fecal coliforms. Additionally, any twigs that are damaged or discolored should be discarded, as they are more likely to be contaminated (Balali *et al.*, 2020). However, if the twigs are not washed properly, or if they are not handled carefully during processing, the risk of contamination can increase. Therefore, it is important to ensure that proper processing techniques are followed to reduce the risk of fecal coliforms contamination.

2.5.2 Educational status

The educational status of farmers has been identified as a significant factor affecting the level of fecal coliform contamination in Khat twigs during cultivation and harvesting (Leight, Crump & Hood, 2018). Farmers with higher educational status have been shown to have a lower level of fecal coliform contamination in Khat twigs during cultivation and harvesting (Amare, Dobo & Haile, 2023). Hassan, Gunaid and Murray Lyon (2018)

study found that the educational status of khat farmers was associated with the level of fecal coliform contamination of khat twigs. Khat twigs harvested by farmers with less education were more likely to be contaminated with fecal coliforms than khat twigs harvested by farmers with more education.

Farmers with less education may be less aware of the importance of good hygiene practices, such as washing their hands before handling khat twigs (Regassa & Regassa, 2018). They may also be less likely to have access to clean water, which can be used to wash khat twigs. A study by Mossie, Kebedez and Gobena (2020) in Ethiopia found that khat twigs from farms where the farmers had less education were more likely to be contaminated with fecal coliforms. This is likely because farmers with less education may not be as aware of the importance of good hygiene practices, such as washing their hands before handling the twigs.

Educational interventions may be an effective way to reduce the level of fecal coliform contamination of khat twigs (Ripanda *et al.*, 2021). These interventions could include training farmers on good hygiene practices and providing them with access to clean water. Education provides farmers with knowledge about proper hygiene practices, such as handwashing after using the bathroom or before handling crops, which can significantly reduce the risk of fecal contamination (Park *et al.*, 2013). Additionally, educated farmers are more likely to use appropriate fertilizers that do not contain human waste products that could further contaminate their crops.

2.5.3 Transportation

Transporting and storing Khat twigs is a crucial aspect of the Khat trade. The poor educational status of middlemen and transporters plays a significant role in the contamination of these twigs with fecal coliforms during transportation and storage (Omar & Mbugua, 2019). The improper handling practices by these players can lead to

cross-contamination between different batches of Khat, further exacerbating health risks for consumers (Al Moaleem *et al.*, 2020). Moreover, low levels of awareness about sanitation standards among transporters may result in suboptimal storage conditions. Poor sanitary conditions in transit vehicles can also worsen this problem; as vehicles move through dusty roads or become contaminated with various contaminants along their routes. The presence of fecal coliforms on Khat has been identified as an issue that poses public health hazards such as gastroenteritis infections from Salmonella organisms (Abdelhafiz *et al.*, 2021).

Ratnadass and Sarter (2023) suggested that awareness campaigns on good agricultural practices and proper handling methods can improve the educational status of farmers while also reducing fecal coliform contamination in the twigs. This approach seems logical since educating farmers about the importance of following specific guidelines when handling their crops can help them avoid common mistakes that often lead to reduced yields or even crop failures. Additionally, by improving their knowledge base through education programs focused on agricultural best practices, farmers may have a better understanding of how to maximize yield potential from their land while minimizing environmental impact.

2.6 Availability of Sanitation Facilities Such as Toilets

The availability of sanitation facilities, such as toilets, can be associated with levels of fecal coliforms contamination of *C. edulis* twigs at different stages of the value chain (Syomiti *et al.*, 2020). Exposure to fecal matter can occur due to breakdowns across a sanitation value chain, which includes the containment of excreta and fecal sludge (Nasim *et al.*, 2023). Sanitary and hygienic requirements for foodstuffs are in place to ensure that they meet physiological human needs and comply with safety standards.

The level of fecal coliforms in khat twigs is significantly higher in areas where there was a lack of sanitation facilities (Amare, Dobo & Haile, 2023). The level of fecal coliforms increased as the khat twigs moved through the value chain, from the farm to the market. This is because khat twigs are often handled by people who do not have access to toilets. They may also be stored in unsanitary conditions, such as on the ground or in open containers. This can lead to the contamination of the twigs with fecal coliforms (Nthiga, 2022).

The availability of sanitation facilities is an important factor in reducing the risk of fecal coliforms contamination of khat twigs (Jones, Bouzid, Few, Hunter & Lake, 2020). When people have access to toilets, they are less likely to defecate in public places. This can help to prevent the contamination of khat twigs with feces.

In addition, when khat twigs are stored in sanitary conditions, the risk of fecal coliforms contamination is reduced. This can be done by storing the twigs in closed containers or in refrigerated environments (Ripanda *et al.*, 2021).

2.7 Irrigation Water Source

The water source associated with the fecal coliforms contamination of *C. edulis* twigs at different stages of the value chain is the water used to irrigate the khat plants (Dongzagla, Jewitt & O'Hara, 2021). This water can be contaminated with fecal coliforms from a variety of sources, including human and animal waste, runoff from agricultural fields, and sewage treatment plants. The risk of fecal coliforms contamination is highest during the early stages of the khat value chain, when the plants are being irrigated (Gizachew, Admasie, Wegi & Assefa, 2020). This is because the plants are still young and their roots are shallow, making them more susceptible to contamination from the water. As the plants grow older, their roots become deeper and they are less likely to be contaminated by the water. However, even mature khat plants can be contaminated by fecal coliforms if

the water used to irrigate them is contaminated. This is because the water can splash onto the twigs of the plants, or it can be carried into the soil by insects or other animals (Berihun *et al.*, 2023).

Once the khat twigs are harvested, they can be contaminated with fecal coliforms if they are handled by people who have not washed their hands properly. This is because fecal coliforms can be present on the skin of people who have used toilets or who have been in contact with animals (Ripanda *et al.*, 2021). Therefore, it is important to use clean water to irrigate khat plants and to wash hands thoroughly before handling khat twigs (Amenu *et al.*, 2021). This helped to reduce the risk of fecal coliforms contamination and protect the health of people who consume khat. Water shortage can increase the risk of fecal coliform contamination of khat twigs. This is because when there is a water shortage, farmers may resort to untreated wastewater to irrigate their khat crops (Tsehaye *et al.*, 2022). Untreated wastewater can contain high levels of fecal coliforms.

The risk of fecal coliform contamination is also higher at certain stages of the khat value chain (Gabriël, Dorny, Saelens & Dermauw, 2022). The risk is higher during the harvesting and processing stages, when the khat twigs are more likely to come into contact with human or animal feces (Nthiga, 2022). If khat twigs are stored in a humid environment, they may become contaminated with bacteria (Nthiga, Mbugua & Anjiri, 2019). This can also increase the risk of fecal coliform contamination.

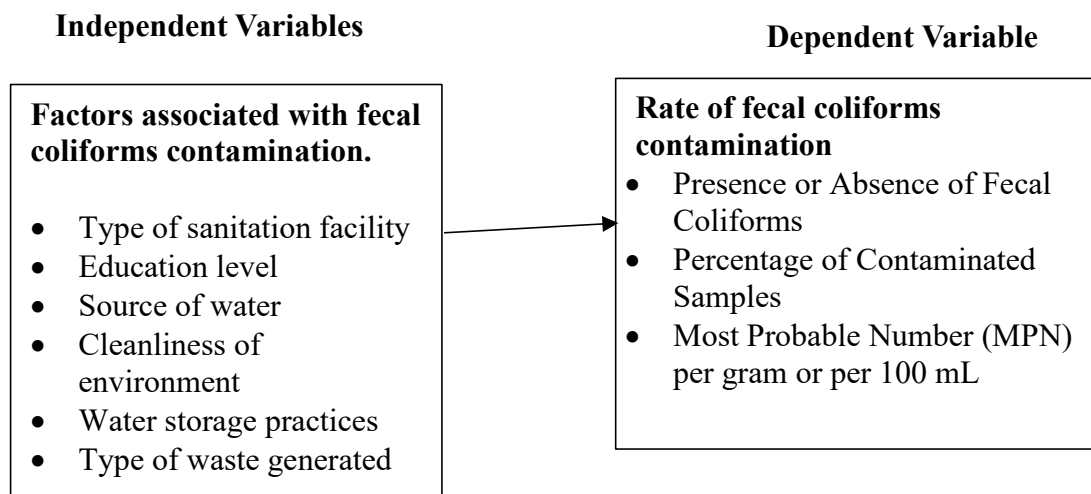
2.8 Conceptual Framework

This study was guided by a conceptual framework that illustrates the relationship between the independent variables and the dependent variable. The dependent variable in this study is the rate of fecal coliform contamination, which is measured using indicators such as the Most Probable Number (MPN) per gram or per 100 mL, the presence or absence of fecal coliforms in the sample, and the Percentage(%) of contaminated samples. These measurements provide a clear indication of the microbial quality of the samples under investigation as shown as in Figure 2.1.

Figure 2.1:

Conceptual Framework (Literature review, 2024)

The study was based on a conceptual framework showing the relationship between the independent and dependent variables



Source: Researcher (2024)

The independent variables include a range of environmental, behavioral, and socio-demographic factors that are believed to influence fecal contamination levels. These factors include gender, age, education level, type and availability of sanitation facilities, handwashing practices, sources of water used, water storage methods, the cleanliness of the immediate and surrounding environment, as well as the types and management of

waste. The conceptual framework assumes that poor sanitation, unsafe water sources, inadequate hygiene practices, and unhygienic environmental conditions can significantly contribute to higher levels of fecal coliform contamination.

By exploring the association between these independent variables and the rate of contamination, the study seeks to identify the critical factors that contribute to microbial contamination, particularly along the value chain of *Catha edulis* (khat) or in the context of water and food handling practices. This framework helped in shaping the research design, formulating the research questions and objectives, and guiding the process of data collection and analysis. It provides a logical basis for understanding not only the extent of contamination but also the underlying causes, thereby supporting the development of targeted interventions to reduce the risk of exposure to fecal pathogens.

CHAPTER THREE: RESEARCH MATERIALS AND METHODS

3.0 Introduction

This chapter describes the methodology used to assess fecal coliform contamination in khat twigs. It details the location of study, study population, research design and sample size. The chapter outlines the laboratory procedures used for microbial analysis, particularly for isolating and identifying fecal coliforms. It also includes descriptions of data collection tools, ethical considerations, and statistical methods employed for data analysis. This chapter ensures transparency and reproducibility of the research process.

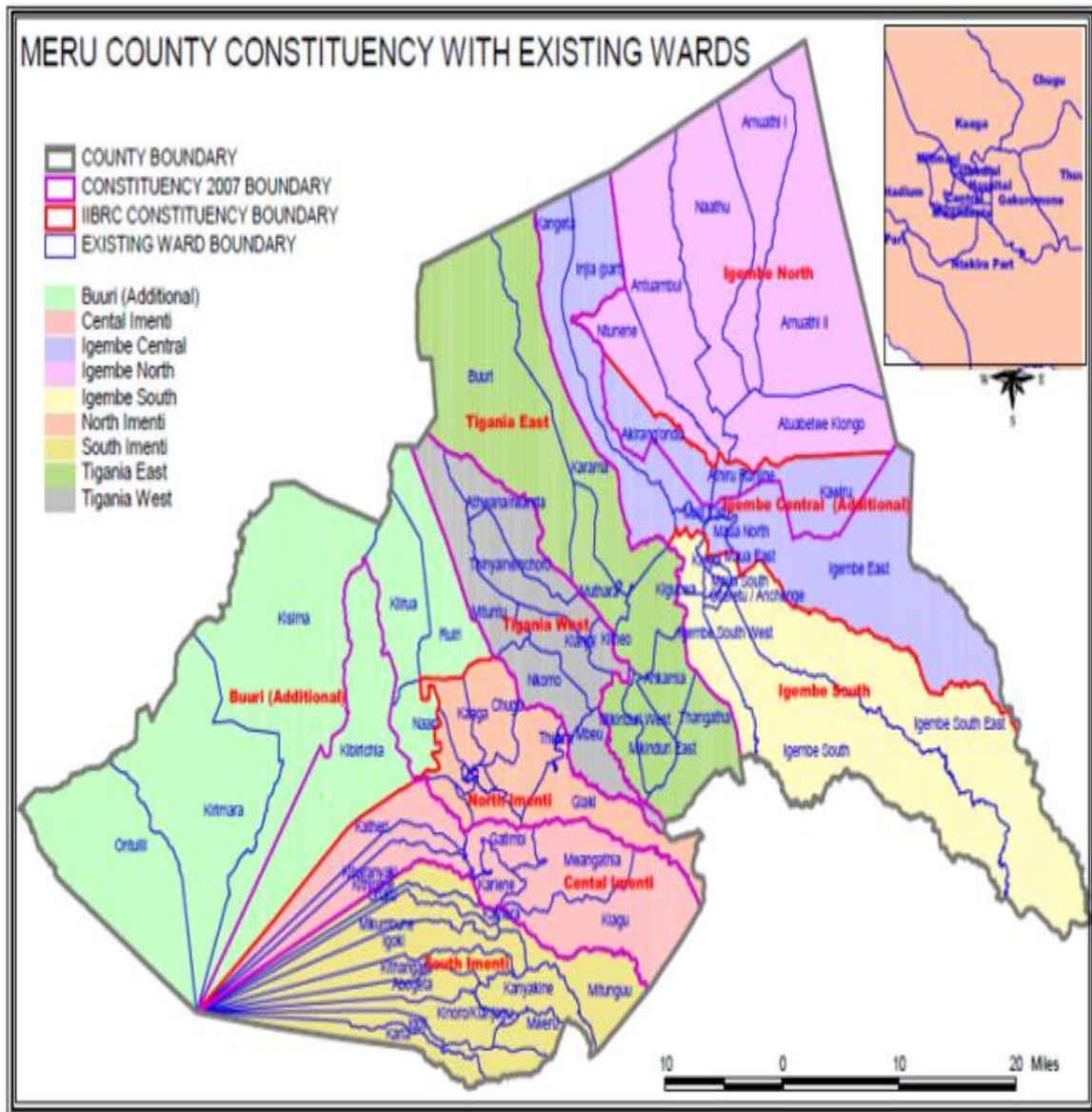
3.1 Location of Study

The study was carried out in Meru County particularly Igembe South Sub County where Khat plant is mostly grown. According to Miraa Growers and Traders Cooperative Union report (2022) there are 1,000 miraa farmers operating profitably in Igembe South Sub County. Igembe South covers an area of 255.2 km². It lies within latitudes 0° 5' and 0° 40' North, and longitudes 37° 50' E and 38° 25' E of the Greenwich Meridian. Altitude ranges from 600m-2,145m above sea level.

The population of Igembe South is 161,646 as per census 2019. It is also a parliamentary constituency, established for the 1988 elections and known as Igembe Constituency before the 2007 elections. Igembe South is one of nine constituencies in Meru County. The sub county has the largest land area in Meru County and is known for its high population density, which is attributed to the high fertility of the land for farming.

Figure 3.1:

Map of Igembe South Sub-County



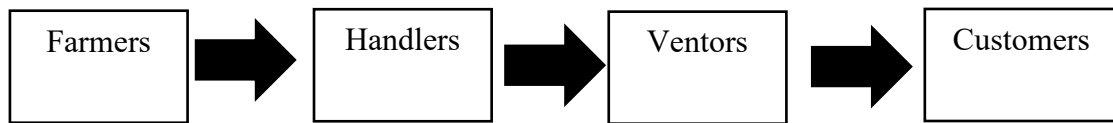
Source: County Government of Meru, (2023)

3.2 Study Population

The target population for this study was formulated by identifying key stakeholders involved in the khat value chain within Igembe South Sub-County, Meru County, Kenya. These included khat farmers, handlers, vendors, and users all of whom play a role in the production, distribution, or consumption of *Catha edulis*.

Figure 3.2 :

Khat Value chain



Source: Growers and Traders Cooperative Union report, (2022)

The study population consisted of 430 khat farmers, 795 handlers, 318 vendors, and 281 customers, bringing the total target population to 1,824 respondents. The target population was 1824 respondents as distributed in Table 3.1.

Table 3.1:*Target Population*

No	Location	Farmers	Handlers	Vendors	Customers	Total
1.	Akachiu	16	30	12	11	69
2.	Antubeiga	23	43	17	15	98
3.	Antubochiu	12	22	9	8	51
4.	Athi	16	29	12	10	67
5.	Athiru Gaiti	19	34	14	12	79
6.	Giika	8	15	6	5	34
7.	Kabuline	16	29	12	10	67
8.	Kangeta	20	37	15	13	85
9.	Kantihari	20	37	15	13	85
10.	Kanuni	15	27	11	10	63
11.	Kiegoi	15	27	11	10	63
12.	Kiengu	26	48	19	17	110
13.	Kiguru	13	23	9	8	53
14.	Kindani	8	16	6	5	35
15.	Kirimampio	17	32	13	11	73
16.	Kithetu	10	19	7	7	43
17.	Luluma	1	1	1	1	4
18.	Makululu	19	37	15	13	84
19.	Maua	29	54	21	19	123
20.	Miori	19	35	14	12	80
21.	Muringene	24	44	17	16	101
22.	Nduguto	11	21	8	7	47
23.	Nguyuyu	23	42	17	15	97
24.	Njia	36	67	27	24	154
25.	Nkinyanga	14	26	10	9	59
Total		430	795	318	281	1824

*Source: Khat farmers (Growers and Traders Cooperative Union report (2022));
Handlers (KNBS 2019); Vendors (KNBS 2019); and Customers (NACADA 2022)*

3.3 Materials

This section outlines the materials used for sample collection, microbiological analysis, and antimicrobial susceptibility testing of fecal coliform isolates from miraa twigs sampled along the value chain in Igembe South Sub-County.

3.3.1 Sampling materials

For the collection of khat twig samples, sterile polyethylene sampling bags were used to ensure aseptic storage and transportation of specimens. To minimize the risk of external contamination, sterile gloves were worn throughout the collection process, while sterile forceps and scissors facilitated the careful cutting and handling of twigs. Each sample was clearly identified using permanent markers and labeling tags, which allowed accurate coding and traceability. The collected samples were immediately placed in cool boxes containing ice packs to maintain a cold chain at approximately 4°C, thereby preserving microbial integrity until laboratory processing. In addition, field data collection sheets were employed to document critical information such as location, date, and the specific stage of the value chain at which the sample was obtained.

3.3.2 Microbiological analysis materials

The microbiological analysis of fecal coliforms required the use of sterile dilution bottles and test tubes for preparing serial dilutions of the samples. Presumptive testing for coliforms was conducted using MacConkey broth and Lauryl Sulfate Tryptose (LST) broth, while confirmatory tests were performed using EC broth incubated at 44.5°C. Petri dishes provided the medium for culture growth, and a membrane filtration unit was utilized for the quantification of coliform bacteria in diluted suspensions. To support bacterial growth at both presumptive and confirmatory stages, incubators were set at 37°C and 44.5°C respectively. Accurate transfer of samples during analysis was achieved through the use of sterile pipettes (1 mL and 10 mL) together with sterile

pipette tips, which minimized contamination and ensured precision throughout the laboratory procedure.

3.3.3 Antimicrobial susceptibility testing materials

The antimicrobial susceptibility testing was carried out using Mueller-Hinton Agar (Liofilchem, Roseto degli Abruzzi, Italy), which is the standard medium recommended for the Kirby–Bauer disc diffusion method. A set of antibiotic discs was employed to represent commonly used drugs in both human and veterinary medicine, including Ampicillin, Ceftriaxone, Doxycycline, Amoxyline, and Azithromycin. To achieve even inoculation of bacterial suspensions onto the agar plates, sterile swabs were utilized under aseptic conditions. Standardization of the bacterial inoculum was ensured using McFarland turbidity standards (0.5), which provided consistency in bacterial density across all test plates. Sterile forceps were used for careful placement of antibiotic discs on the inoculated plates, while incubation was performed at 35–37°C for 18–24 hours to allow observable growth inhibition patterns. The diameters of the inhibition zones were measured using a calibrated ruler or digital caliper in millimeters, and the results were interpreted following Clinical and Laboratory Standards Institute (CLSI) guidelines.

3.3.4 Personal protective equipment (PPE)

To safeguard both the researcher and the integrity of the laboratory procedures, personal protective equipment (PPE) was employed at all times. This included laboratory coats, sterile gloves, protective face masks, and safety eyewear to prevent exposure to pathogenic organisms and minimize the risk of cross-contamination. All reagents and laboratory materials were sourced from accredited suppliers and were either pre-sterilized or sterilized through autoclaving at 121°C for 15 minutes before use. Furthermore, quality control procedures were strictly adhered to by incorporating

standard reference strains, such as *Escherichia coli* ATCC 25922, which served as benchmarks for validating the accuracy and reliability of susceptibility testing.

3.4 Research Design

This study adopted a cross-sectional descriptive study design involving one-time sampling of khat (*C. edulis*) at the pre-harvest stage, during harvesting, delivery, sale point and users.

3.5 Selection Criteria

The study participants were selected from the eligible target population, based on the laid down criterion of inclusion and exclusion to avoid bias.

3.5.1 Inclusion criteria

Khat farmers, handlers, vendors and users from the study site and those who gave informed consent were included in the study.

3.5.2 Exclusion criteria

Khat farmers, handlers, vendors and users who did not consent to participate in the study or those who did not reside in the specified study sites were excluded from the study.

3.6 Sample Size Determination

This study used Yamane's formula, to determine the sample size of the population as show as in equation 3.1.

$$n = \frac{N}{1+N(e^2)} \quad (3.1)$$

Where; n = the sample size,

N = the population size,

e= the acceptance sampling error give at 0.05

$$n = 1824 / (1 + 1824 \times 0.05^2)$$

$$n = 328$$

Therefore, the sample size was 328 respondents distributed as shown in Table 3.2.

3.6 Sampling Techniques

The study adopted multistage sampling. The researcher stratified respondents into 25 strata representing each of the 25 locations. The researcher employed a stratified sampling technique to administer 328 research instruments to a diverse group of respondents, including farmers, handlers, vendors, and consumers. The data collection method was utilized to mitigate any potential bias from student respondents, ensuring that each individual in the population had an equitable chance of being selected as a sample, thus representing the entire population. This study further use stratified sampling to put respondents into four strata (farmers, handlers, vendors and customers). The researcher then picked respondents proportionately from each stratum. Simple random sampling was used to select a respondent from each strata to participate in the study (Table 3.2). This sampling technique provided each member with an opportunity to participate in the study.

Table 3.2:

Sample Size (N=328)

No.	Location	Farmers	Handlers	Vendors	Customers	Total
1.	Akachiu	3	5	2	2	12
2.	Antubeiga	4	8	3	3	18
3.	Antubochiu	2	4	2	1	9
4.	Athi	3	5	2	2	12
5.	Athiru Gaiti	3	6	3	2	14
6.	Giika	1	3	1	1	6
7.	Kabuline	3	5	2	2	12
8.	Kangeta	4	7	3	2	16
9.	Kantihiari	4	7	3	2	16
10.	Kanuni	3	5	2	2	12
11.	Kiegoi	3	5	2	2	12
12.	Kiengu	5	9	3	3	20

13.	Kiguru	2	4	2	1	9
14.	Kindani	1	3	1	1	6
15.	Kirimampio	3	6	2	2	13
16.	Kithetu	2	3	1	1	7
17.	Luluma	1	1	1	1	4
18.	Makululu	3	7	3	2	15
19.	Maua	5	9	3	3	20
20.	Miori	3	6	3	2	14
21.	Muringene	4	7	3	3	17
22.	Nduguto	2	4	1	1	8
23.	Nguyuyu	4	8	3	3	18
24.	Njia	6	11	4	5	26
25.	Nkinyanga	3	5	2	2	12
	Total	77	143	57	51	328

Source: Researcher, (2023)

3.7 Research Instruments

The study collected data using questionnaires and data sheets (Appendix C to F)

3.8 Pilot Study

The piloting of the questionnaires refers to a study conducted before main study. This was done to ascertain the reliability and validity of the research instrument as recommended by (Sreevidya & Sunitha, 2011). For research instruments to be tested for validity and reliability using the test-retest method, pilot testing was an essential prerequisite (Mugenda & Mugenda, 2008). Additionally, the pilot study helped identify any inadequacies or complexity with the suggested methods and instruments and identified any practical issues with the research procedures. The pilot testing was conducted in Nchiru area of Meru County which has similar characteristics with Igembe South Sub County.

3.8.1 Validity of reseach instruments

Validity pertains to the extent to which an instrument accurately assesses the intended construct. It also pertains to the precision and significance of deductions or the extent to which the results of data analysis elucidate the phenomenon being studied (Bahari, 2010). The study identified three primary forms of validity for the instruments: face validity, content validity, and construct validity. The researcher established the face validity of the instruments by ensuring that the questionnaires were designed in accordance with the conceptual framework depicted in Figure 2.1.

This was done to encompass all the study variables and sub constructs. Content validity refers to the extent to which the data collection instrument produces statistically expected results in a study (Collis & Hussey, 2014). The researcher conducted a comprehensive review of the existing literature to ascertain the necessary components for measuring the constructs. The researcher sought guidance from his supervisors regarding the content validity of the study instruments. Their perspectives and recommendations were utilized to enhance the pertinence, lucidity, and phrasing of the items or inquiries in the research tool. Construct validity aims to determine the degree to which a research instrument can be considered a reliable measure of a specific characteristic or quality. Construct validity refers to the extent to which theories or ideas have been accurately and effectively translated into concrete measures or assessments. The researcher confirmed the construct validity of the instruments by examining both theoretical and empirical literature.

3.8.2 Reliability of reseach instruments

Reliability refers to the extent to which a particular test can consistently produce identical results or scores when administered to the same individuals on multiple occasions. In order for research to have internal coherence, estimates of reliability are determined by calculating the average inter-correlations among all the individual items

within a study (Greene, 2012). Following the pilot study, the Cronbach's Alpha coefficient was calculated for the items or questions in the questionnaires using SPSS version 24 software. Cronbach's Alpha was employed to ascertain the reliability coefficient of a set of research variables by assessing the intercorrelation among the items in the questionnaires. A Cronbach's Alpha coefficient of 0.7 or higher indicates a high level of internal consistency in the study questionnaire (Mugenda & Mugenda, 2008).

$$\alpha = \frac{Nc}{v+(N-1)c} \quad 3.2$$

Where; α represents an internal consistency; v represents average covariance; N represents number of items and c represents average variance.

3.9 Data Collection Techniques

3.9.1 Sample collection procedure

The collection of *C. edulis* from the study site was conducted with the assistance of two research assistants who were involved in all stages of the Khat value chain. An average of 300g of khat twigs per site were obtained and stored in cool, dry, and aerated bags during transportation. A confidential questionnaire, assigned a unique identifier, was administered to each participant in a manner that ensured their comfort and comprehension. The questionnaire aimed to gather bio-data, including information on the source of *C. edulis* twigs, the location of respondents, and their level of public health awareness regarding infection transmission. Additionally, it sought to gather data on the availability of social amenities such as sewerage, toilets, and access to food and water.

3.9.2 Sampling protocol for khat twigs

A pre-labeled Produce Sample Collection and Processing Form was retrieved and the date and time of the sampling location were recorded. The labeled Whirl-Pak plastic bag

was opened by delicately extracting the tabs on the side of the bag without making contact with the opening or interior of the bag. Subsequently, 300 grams of Khat twigs were inserted into the bag and then sealed. The whirl-pak bag containing the samples was promptly placed in the ice chest along with an ice pack. It was then transported to the laboratory within a time frame of 6 hours and stored in a refrigerator set at a temperature of 4°C until it is time for analysis (Corrales *et al.*, 2023).

3.9.3 Sample processing to enumerate fecal coliforms

Outside Whirl-Pak bag was sprayed with 70% alcohol to get rid of any contaminant that might have come into contact with the surface of the bag. The Whirl-Park bag was then opened and 500ml of sterile water added, then sealed and shaken vigorously afterwards. This was to rinse of any fecal coliform contamination from the surface of khat twigs. The twigs were then removed from the Whirl- Pak bag and rinsed bacteria stored at 4°C until when they are ready for processing.

3.9.4 Culturing of fecal coliforms

Serial dilution (up to 2-fold i.e. 10⁻¹ and 10⁻²) of the original suspension of coliforms in Whirl bag was done and 100 micro liters from each dilution factor were spread on Macckonkey agar using the glass spreader and incubated at 37°C for 24 hours after which the colonies (pink/red) were counted and the number obtained was used to determine the Colony Forming Units per gram of the sample.

3.9.5 Antimicrobial susceptibility testing

To isolate the single colony of *E.coli* to be used in antibiotic susceptibility test, one loop of the pink/red colonies enumerated were suspended in 1ml of sterile distilled water and plated on selective media (EMB Levine agar, Liofilchem, Roseto, Italy) and the isolate was identified according to its appearance (green metallic sheen).

Following established protocols in microbiology, antibiotic susceptibility was assessed by disk diffusion. In a nutshell, MuellerHinton agar (Liofilchem, Roseto, Italy) was used to plate bacterial suspensions from the selective EMB media (0.5 turbidity, McFarland Standard). Following the addition of disks containing antibiotics, the plates were incubated at 37°C for 24 hours. Antibiotic resistance and susceptibility were determined by meticulously measuring the diameters of the growth inhibition zones(Appendix F).

3.10 Data Processing, Analysis and Presentations

The demographic data, water source information, and hygiene practices were recorded on raw data forms and then transferred to an Excel spreadsheet. The accuracy of the data was verified before entering it. The data was subsequently analyzed using the statistical package of social sciences (SPSS) version 23.0. The t-test was employed to assess the disparity in the rate of fecal coliforms contamination of *C. edulis* twigs at different stages of value chain. The measurement of the zone of inhibitions, factors associated with the fecal coliforms contamination of *C. edulis* twigs at different stages of value chain and gender difference in the rate of fecal coliforms contamination of *C. edulis* twigs. The data findings were presented using various visual aids such as tables, charts, and graphs, in addition to written explanations. Percentage(%)s were employed to delineate the attributes of the examined population, encompassing the occurrence of IPI in each subgroup, age cohorts, and gender. The statistical significance level was set at $P < 0.05$ for the dependent variable in relation to the independent variable. The prevalence of fecal coliforms served as the dependent variable, while the socio-demographic factors (age and sex), source of water, estate, and environmental sanitation served as the independent variables. The statistical significance level was set at $P < 0.05$.

3.11 Logistical and Ethical Considerations

The researcher ensured that he had access to the necessary equipment and expertise to accurately measure fecal coliforms and other microbial indicators of contamination. The researcher considered the logistics of sample collection and transportation, ensuring that samples were collected at the appropriate stages of the value chain and transported to the laboratory in a timely and safe manner. The researcher obtained ethical approval from an institutional review board of Ethics from Meru University before conducting the study. Prior to giving their consent to participate in the research, the respondents were provided with comprehensive information regarding the overall objective of the study (Appendix B). Additionally, any potential factors that could influence their willingness to participate were thoroughly explained. The participants were guaranteed that their rights and privacy would be maintained throughout the duration of the study. The researcher adhered to ethical principles by ensuring the participants' safety and avoiding any potential harm that could arise from their involvement in the study. The research strictly adhered to the principle of voluntary participation.

CHAPTER FOUR: RESULTS AND DISCUSSIONS

4.0. Introduction

This chapter provides an exposition of the outcomes, discoveries, and deliberations. The arrangement of this chapter was determined by the objectives. The study began by analyzing the return rates and characteristics of the respondents in simple random sampling, stratified sampling, snowball samplings, and purposive sampling. It then proceeds to assess the presence of fecal coliforms in *C. edulis* twigs along the value chain in Igembe south sub-county, Meru, Kenya.

4.1 Response Rate

Out of the 328 questionnaires distributed 322 were returned. Also, out of 328 intended samples of Khat twigs 322 (98.2%) were collected. The high response rate was achieved due to researcher follow up of the respondents and clarification any question. Table 4.1 indicates the response rate.

Table 4.1:

Response rate (N=328)

Responses	Questionnaires		Khat samples	
	Frequency(n)	Percentage(%)	Frequency(n)	Percentage(%)
Administered	328	100	328	100
sample				
Collected	322	98.2	322	98.2
Not collected	6	1.8	6	1.8

Source: (Researcher, 2023)

4.2 Level of Fecal Coliforms Contamination of *C. Edulis* Twigs at Different Stages of Value Chain

The study sought to assess the rate of fecal coliforms contamination of *C. edulis* twigs at different stages of value chain. The number of colonies counted were converted into colony forming units. The colony forming units values were converted into log 10 hence the generating the means. The average fecal coliform contamination levels were highest for handlers (4.0852) and lowest for customers (3.9756). The variability in fecal coliform contamination levels is highest for farmers (0.18409) and lowest for customers (0.16827) as detailed on Table 4.2.

Table 4.2:

Fecal califorms contamination of khat (N=322)

	N	Mean	Std. Deviation	Minimum	Maximum
Farmers	74	3.9690	.18409	3.67	4.57
Handlers	140	4.0852	.17678	3.68	4.50
Vendors	57	3.9858	.15413	3.66	4.40
Customers	51	3.9756	.16827	3.73	4.46

Source: (Researcher, 2023)

Data showed that different sectors of food handlers demonstrate varying average fecal coliform contamination levels in their work activities. The mean contamination levels reveal that handlers have the highest value at 4.0852 and customers possess the lowest value at 3.9756. Food supply chain contamination is most likely to occur during the first stages since handlers display a higher contamination level than other groups. The contamination levels among farmers demonstrate the most significant variability since they attained a standard deviation value of 0.18409. The contamination levels demonstrated by customers exhibit minimal variability because they average at 0.16827

compared to other groups. This data suggests an interesting pattern in fecal coliform contamination levels across different groups involved in food handling. The fact that handlers have the highest average contamination levels while customers have the lowest is significant. It implies that contamination tends to occur more frequently at the earlier stages of food production and handling, which is not entirely surprising given the various points of contact and potential for mishandling during these stages.

However, what's particularly noteworthy is the variability in contamination levels among different groups. The variability being highest for farmers and lowest for customers raises some intriguing questions. One possible explanation could be the difference in practices and standards across these groups. Farmers might operate under varying levels of hygiene protocols and environmental conditions, leading to greater fluctuations in contamination levels. On the other hand, customers, being the end consumers, are more removed from the direct handling and production processes, hence experiencing lower variability.

The study findings are in line with Okpala and Korzeniowska (2023) findings that variability of fecal coliform contamination levels underscores the importance of implementing consistent and stringent hygiene measures, especially at the primary stages of food production. It also emphasizes the need for effective monitoring and regulation throughout the food supply chain to ensure food safety standards are maintained. Additionally, educating and training handlers and farmers on proper hygiene practices can help mitigate contamination risks and ultimately safeguard public health

Figure of variability are the standard deviation of fecal coliform contamination levels among the different groups (farmers, handlers, and customers). Standard deviation is a measure of how spread out the contamination levels are within each group. These results concur with a study done by Gezie in the year (2019) where he attributed the higher

variability among farmers to factors such as agricultural practices, environmental conditions, and handling methods.

The minimum and maximum values for fecal coliform contamination showed similar patterns across all stages, with the lowest minimum value being 3.66 for farmers and the highest maximum value being 4.57 for handlers. This aligns with the study by Daly and Harris, (2022) that noted that the similarity in minimum and maximum contamination values across different stages of the distribution chain may reflect common sources of contamination and similar environmental exposures throughout the supply chain.

The F-statistic is a measure used in analysis of variance (ANOVA) to determine whether there are statistically significant differences between the means of three or more groups. From table 4.3 indicated that the F-statistic (10.539) is significant ($P=0.000$) thus affect Value Chain on fecal coliform contamination. The F-statistic of 10.539 indicates that there is a significant effect of value chain on fecal coliform contamination. The P-value associated with the F-statistic is extremely low ($P=0.000$), indicating that the likelihood of observing such a result by random chance alone is essentially zero. This strengthens the evidence for rejecting the null hypothesis, which suggests that there are no differences in fecal coliform contamination levels across the different stages of the value chain.

This is in line with the study done by Ahmedsham *et al.* (2018) that indicated that microbial contamination levels vary significantly throughout the distribution chain, indicating potential differences in hygiene practices, environmental exposures, and handling methods at each stage. However, this analysis does not tell which specific stages differ from each other. Therefore, the study performed post-hoc tests (Tukey's HSD) for pairwise comparisons as shown in Table 4.3.

Table 4.3:*ANOVA results of fecal coliform contamination levels(N=322)*

	Sum of Squares	Df	Mean Square	F	p
Contrast	.951	3	.317	10.539	.000
Error	9.564	318	.030		

*Source: (Researcher, 2023)***Table 4.4:***Tukey HSD of fecal coliform contamination levels (N=322)*

(I) Value Chain	(J) Value Chain	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Farmers	Handlers	-.1162*	.02492	.000	-.1806	-.0519
	Vendors	-.0169	.03056	.946	-.0958	.0621
	Customers	-.0067	.03156	.997	-.0882	.0749
Handlers	Farmers	.1162*	.02492	.000	.0519	.1806
	Vendors	.0994*	.02725	.002	.0290	.1697
	Customers	.1096*	.02836	.001	.0363	.1828
Vendors	Farmers	.0169	.03056	.946	-.0621	.0958
	Handlers	-.0994*	.02725	.002	-.1697	-.0290
	Customers	.0102	.03343	.990	-.0761	.0965
Customers	Farmers	.0067	.03156	.997	-.0749	.0882
	Handlers	-.1096*	.02836	.001	-.1828	-.0363
	Vendors	-.0102	.03343	.990	-.0965	.0761

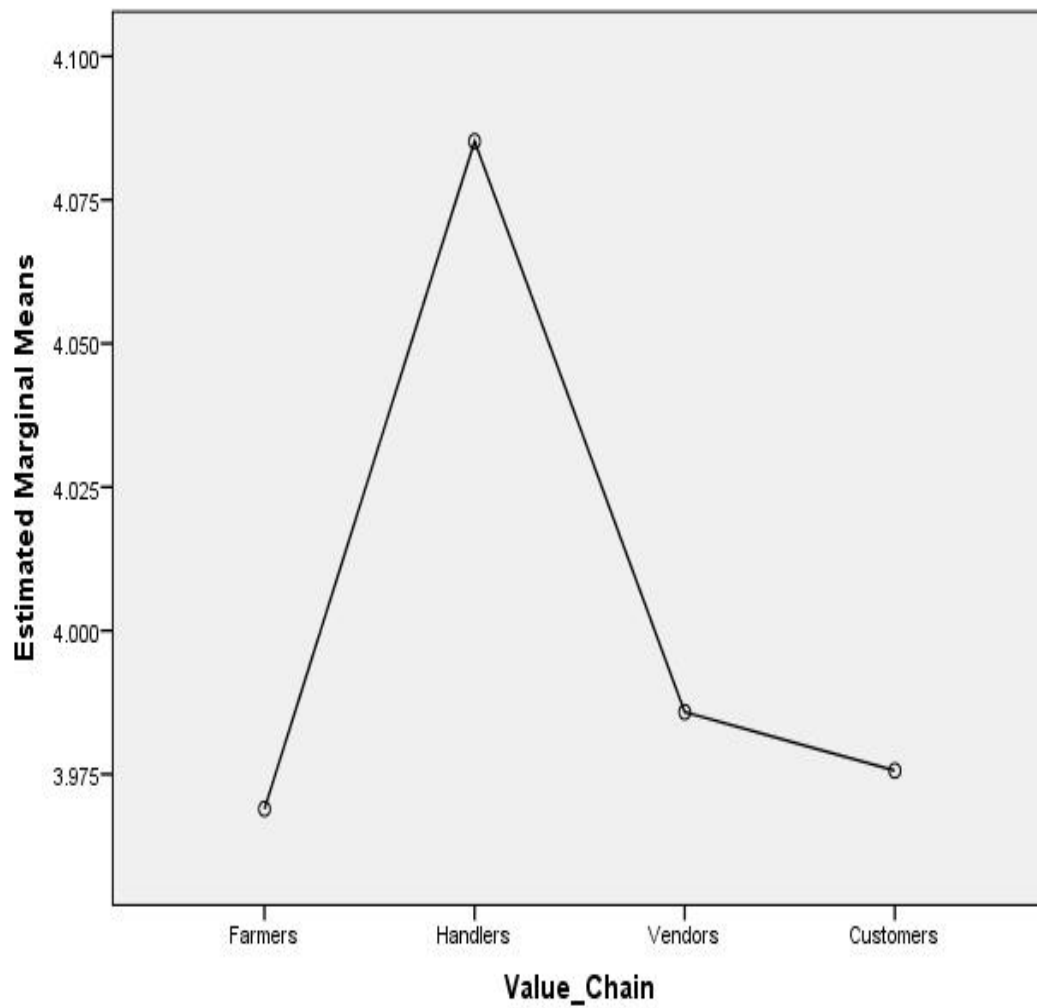
*Based on observed means. The error term is Mean Square(Error) = .030;***. The mean difference is significant at the .05 level;**Source: (Researcher, 2023)*

Study findings from Tukey HSD revealed that farmers with mean difference=0.0169; $p=0.000$ had significantly lower fecal coliform contamination than handlers (mean difference=0.1162; $p=0.000$), vendors (mean difference=0.0994; $p=0.002$), and customers (mean difference=0.1096; $p=0.001$). This agrees with the study done by Harris *et al.* (2018) that found that farmers consistently exhibited lower levels of fecal coliform contamination in comparison to handlers, vendors, and customers. Handlers have significantly more contamination than customers (marked with *). A study by Nthiga (2022) supported the finding that handlers have significantly higher contamination levels compared to customers in the context of *C. edulis*) distribution. There was no significant difference between farmers and vendors, or between handlers and vendors. The study by Roth *et al.* (2018) found that both farmers and vendors exhibited similar levels of fecal coliform contamination, suggesting no significant difference between these groups.

All other comparisons with farmers or customers had a P-values exceeding 0.05, meaning there's no significant difference. These results suggest that fecal coliform contamination increases as Khat twigs move through the value chain, with the highest levels found in customers. This highlights the need for improved hygiene practices at all stages, especially for handlers and vendors. This study aligns with the finding by Zerbe (2022) that noted that fecal coliform contamination increased as *C. edulis* twigs move through the value chain, emphasizing the importance of implementing enhanced hygiene protocols and quality control measures at all stages of the distribution process.

Figure 4.1:

Estimated marginal means of fecal coliforms contamination



Source: Researcher, (2023)

4.3 Descriptive Results of Factors Associated With the Fecal Coliforms Contamination of *C. Edulis* Twigs at Different Stages of Value Chain

The study conducted descriptive statistics for factors associated with the fecal coliforms contamination of *C. edulis* twigs at different stages of value chain. The responses to these statements were gathered through a questionnaire, as outlined in the following narratives.

4.3.1 Gender of the respondents

Drawing from the data provided in Table 4.5, it is apparent that the largest portion of participants, 255 (79.2%) individuals, were male, while 67 (20.8%) were female. These results suggest that both genders were equally represented in the study, implying a equal distribution of gender among the participants. Garg, Schiebinger, Jurafsky and Zou (2018) stated that it's crucial to assess whether the gender distribution accurately reflects the population being studied and any skewed gender distribution could potentially bias the results.

Table 4.5:

Gender of the Respondents(N=322)

Gender	Frequency(n)	Percentage(%)
Males	255	79.2
Females	67	20.8

Source: (Researcher, 2023)

4.4.2 Age bracket of the respondents

In Table 4.6, 85 respondents (26.4%) were between the ages of 15–35 years. The majority of participants were aged 35–50 years, totaling 140 (43.5%), followed by 97 respondents (30.1%) who were above 50 years old. Overall, it seems that the study had a

relatively balanced distribution across different age groups. Zhao, O'Connor, Wu and Lumpkin (2021) stated that it's essential to consider how demographics of age bracket might influence the study's findings and interpretations, as different age groups may have distinct perspectives, behaviors, and experiences that could impact the results.

Table 4.6:

Age bracket of the respondents (N=322)

Age Bracket (Years)	Frequency(n)	Percentage(%)
15 – 35 Years	85	26.4
35 - 50 years	140	43.5
50 years and above	97	30.1

Source: Researcher, (2023)

4.3.3 Sanitation facility

Table 4.7 shows that 202 (62.7%) indicated that they had a sanitation facility in their respective locations while 120 (37.3%) indicated that they had no sanitation facility in their respective locations. Access to sanitation facilities is crucial for maintaining public health and well-being. Without proper sanitation, individuals are at a higher risk of contracting diseases, and communities may struggle with issues related to hygiene and environmental pollution.

The fact that nearly a third of the surveyed population lacked access to sanitation facilities underscores the need for urgent action to address this issue. Governments, non-profit organizations, and other stakeholders should work together to improve infrastructure and provide adequate sanitation facilities to all communities, especially those in underserved areas. The Findings concur with Mara and Evans (2018) that the efforts to improve sanitation should not only focus on providing access to basic facilities but also on promoting hygiene education and sustainable sanitation practices. This

holistic approach is essential for ensuring that everyone can enjoy their right to sanitation and live in healthy environments.

Table 4.7:

Sanitation Facility (N=322)

Response	Frequency(n)	Percentage(%)
Yes	202	62.7
No	120	37.3

Source: Researcher, (2023)

4.3.4 Type of sanitation facility in each farm

Table 4.8 presents the distribution of sanitation facilities based on their types, with frequencies and corresponding Percentages. The three categories listed are public, private, and open or sharing facilities. The data indicated that open or sharing facilities at 64.6%, followed closely by private facilities constituted the largest portion at 22.4%. Public facilities made up the smallest portion at 13%. The dominance of open or sharing and private facilities suggests a potential disparity in access to sanitation services. The open or sharing facilities imply a more communal or shared arrangement, which might suggest a lack of access to private sanitation facilities for some individuals or communities. On the other hand the private facilities typically refer to those owned or accessed by individuals or households, indicate a level of exclusivity or ownership. The findings concur with Meerow and Newell (2017) that the high Percentage(%) of open or sharing facilities indicate issues related to infrastructure development or urban planning, where communities lack adequate private sanitation facilities, leading to reliance on shared or communal options.

Table 4.8:

Type of Sanitation Facility (N=322)

Type of sanitation facility	Frequency(n)	Percentage(%)
Public	42	13.0
Private	72	22.4
Open or Sharing	208	64.6
Total	322	100.0

Source: Researcher, (2023)

4.3.5 Level of education of the respondents

Majority of the farmers had only very basic primary education recording 55.3%. This is detailed on Table 4.9 where 178 (55.3%) were found to have primary level of education, 124 (38.5%) had secondary level of education 15 (4.7%) had tertiary level of education and 5 (1.6) did not attend any. The fact that 55.3% of respondents surveyed had only primary education suggests that there might be limited access to higher levels of education or perhaps other factors influencing educational attainment within this demographic. The limited education impact farmers' ability to adopt modern farming techniques, understand complex agricultural practices, or engage with new technologies. It also affect their access to information, resources, and markets, which are crucial for improving agricultural productivity and livelihoods.

The findings concur with Siegner, Acey and Sowerwine (2020) that understanding the educational profile of farmers can inform policymaking and resource allocation, guiding efforts to support rural education and empower farming communities. Education, skills and development work towards a more sustainable and inclusive agricultural sector, benefiting both farmers and broader society.

Table 4.9:

Education level of the farmers(N=322)

Education level	Frequency(n)	Percentage(%)
Primary	178	55.3
Secondary	124	38.5
Tertiary	15	4.7
Did not attend any	5	1.6
Total	322	100.0

Source: Researcher, (2023)

4.3.6 Use of irrigation in the khat plantations

The data presented in Table 4.10 suggests that irrigation practices varied among the farmers interviewed regarding their Khat plantations. 168 out of the 322 respondents interviewed (52.2%) indicated that they practiced irrigation in growing the Khat Plant. However 154 (47.8%) of the respondent farmers indicated that they did not practice irrigation in their Khat Plantations. This indicates a significant portion of the farmers were actively utilizing irrigation methods to support the growth of Khat. On the other hand, 154 farmers, constituting about 52.2% of the respondents, stated that they did not employ irrigation techniques in their Khat plantations.

The discrepancy in irrigation practices among the surveyed farmers could stem from various factors such as access to water resources, geographic location, economic constraints, and traditional farming practices. Those who practice irrigation might have access to reliable water sources, such as rivers, wells, or irrigation systems, enabling them to maintain consistent moisture levels for their crops. Conversely, farmers who do not practice irrigation might rely on rainfall or may not have access to the necessary infrastructure for irrigation.

The findings concur Dardonville, Urruty, Bockstaller and Therond (2020) that the diversity in irrigation practices highlights the complexity of agricultural systems and the adaptability of farmers to their local environments. It shows the importance of understanding local contexts and challenges when devising strategies for agricultural development and water resource management.

Table 4.10:

Irrigation in the khat plantations (N=322)

Response	Frequency(n)	Percentage(%)
Yes	168	52.2
No	154	47.8

Source: Researcher, (2023)

4.3.7 Type of water used for irrigation

Most of the farmers used river water, while others irrigated their land using water from wells. Only 16.5% used borehole water, with least recorded among farmers who used treated water sources for irrigating in their land as documented clearly on Table 4.11.

Table 4.11:

Type of water used (N=322)

Type of water used	Frequency(n)	Percentage(%)
River	163	50.6
Borehole	53	16.5
Wells	106	32.9

Source: Researcher, (2023)

Firstly, it's notable that the majority of farmers (50.6%) rely on river water for irrigation. This reliance on river water is understandable given its accessibility and often consistent

supply, especially in agricultural regions. Rivers can be a convenient and natural source of water for irrigation, particularly for those situated close to riverbanks.

Secondly, the mention of farmers using well water highlights another common practice in agricultural areas. Wells can provide a reliable source of water, especially in regions where groundwater is abundant and accessible. However, the availability and quality of well water can vary significantly depending on factors such as depth, location, and geology.

The statistic that only 16.5% of farmers use borehole water suggests that borehole irrigation is less prevalent among the farming community. Boreholes can be a valuable source of water, particularly in areas where surface water may be scarce or unreliable. However, the lower Percentage could indicate challenges such as the cost of drilling and maintaining boreholes, as well as potential issues with water quality or availability.

Lastly, the mention of treated water sources being the least utilized for irrigation is intriguing. Treated water, such as reclaimed or recycled water, is often subject to purification processes to meet certain quality standards. While treated water can be a sustainable option for irrigation, its lower usage among farmers may stem from factors such as limited availability, infrastructure constraints, or perceptions regarding water quality.

Findings by Srivastav *et al.*, (2021) stated that farmers' preferences and practices regarding water sources is essential for promoting sustainable water use in agriculture and ensuring the resilience of agricultural systems in the face of environmental challenges. Also our findings concur with those findings by Torres, Kallas and Herrera (2020) that farmers rely on river water for irrigation due to its accessibility and often consistent supply, especially in agricultural regions situated close to riverbanks.

Research in agricultural productivity often highlights the significance of river water as a primary source for irrigation due to its natural availability and convenience.

4.3.8 Sources from which they access water

Table 4.12 presents data on the various sources from which people obtain water, along with their frequencies and Percentage(%)age(%)s. It appears that there are three primary sources: municipal taps, wells, and water vendors. The data indicates that wells are the most common source of water, accounting for 70.2% of the responses. This could be due to several factors, such as geographic location, infrastructure availability, or cultural practices. Wells are often a traditional and reliable source of water in many regions, especially in rural areas where access to municipal water systems may be limited.

Municipal taps represent the second most common source, with 25.2% of respondents indicating their use. This suggests that a significant portion of the population has access to centralized water infrastructure provided by local governments. Municipal tap water is typically treated and regulated, providing a relatively safe and convenient option for obtaining water. Water vendors, while accounting for a smaller Percentage at 4.7%, still play a significant role in providing water access. This could be particularly relevant in areas where municipal water infrastructure is inadequate or unreliable. Water vendors often sell water from various sources, including municipal supplies or private wells, and their services cater to individuals or communities lacking direct access to piped water.

The findings concur with Vollmer *et al.*, (2018) that the distribution of water sources highlights the diversity of water access scenarios. Understanding the distribution of water patterns is crucial for policymakers and organizations involved in water resource management and infrastructure development to ensure equitable access to safe and reliable water sources for all communities. Access to treated and regulated water, potentially offering a higher level of sanitation and safety compared to other sources.

Table 4.12:

Sources from which they access water (N=322)

Water Source	Frequency(n)	Percentage(%)
Municipal taps	81	25.2
Well	226	70.2
Water vendors	15	4.7

Source: Researcher, (2023)

4.3.9 Cleanliness in their local environment

According to the respondents, the local environment where the Khat plants were placed after harvesting ranged from good (clean environment), to satisfactory which means just preliminary cleaning to bad (very dirty environments). Chances of faecal coliform for the latter two could not be ruled out. Table 4.13 presents the study results of environmental cleanliness after harvesting of the Khat by the farmers.

Table 4.13 presents data on the perceived cleanness of the local environment, categorized into three groups: Good, satisfactory, and Bad. These categories represent varying degrees of cleanliness as perceived by respondents or observers. Firstly, the majority, 91.5%, rated the cleanness of their local environment as "satisfactory," followed by 5.9% who rated it as "Good," and a smaller portion, 2.8%, who rated it as "Bad." The distribution suggests that a significant portion of respondents view their local environment as merely acceptable ("satisfactory"), while a smaller but notable portion view it positively ("Good"). However, there is still a concerning minority who perceive their environment as unclean or "Bad."

The finding by Truong *et al.*, (2017) critique that individuals' perceptions of cleanliness in their local environment are influenced by various factors including aesthetic quality, safety, and maintenance. Another study focusing on urban environments have

highlighted the importance of well-maintained public spaces, effective waste management systems, and green infrastructure in shaping positive perceptions of cleanliness (Ojobo *et al.*, 2024). Additionally, socioeconomic status and cultural background play significant roles in shaping cleanliness perceptions, with residents of higher-income neighborhoods often perceiving their environment as cleaner than those in lower-income areas.

Table 4.13:

Cleanness in their Local Environment(N=322)

Cleanliness	Frequency(n)	Percentage(%)
Good	19	5.9
Satisfactory	294	91.3
Bad	9	2.8

Source: Researcher, (2023)

4.3.10. Type of waste

Different material were observed in these environments. These ranged from stagnant water, animal and human feces among others. There are probability of fecal contamination of Khat through these materials by sanitation related organisms. The data presented in Table 4.14 provides valuable insights into the perceptions of respondents regarding the types of waste found in the environment. It appears that stagnating water is overwhelmingly perceived as a prevalent type of waste, with 41.9% of respondents identifying it as such. This high Percentage(%)age(%) likely reflects the widespread concern over stagnant water's role as a breeding ground for various contaminants and disease vectors.

Table 4.14:

Kind of waste (N=322)

Kind of waste	Frequency(n)	Percentage(%)
Human feces	22	6.8
Stagnation of water	135	41.9
Animal feces	161	50.0
All of these	4	1.2

Source: Researcher, (2023)

Additionally, animal feces are acknowledged by a significant portion of respondents, with 36.4% citing them as a form of environmental waste. This finding underscores the recognition of animal presence and their contribution to environmental sanitation challenges. Surprisingly, human feces are mentioned by only 6.8% of respondents, indicating a comparatively lower level of awareness or concern regarding this type of waste or acknowledging its proper handling. However, it's essential to note that 1.2% of respondents recognize the presence of all three mentioned types of waste, suggesting a subset of the population that is more comprehensive in their understanding of environmental sanitation issues.

The findings from this study also concur with the findings from Magnini and Zehrer (2021) that different nature of environmental waste need interventions to address the various sources of pollution effectively. Efforts to raise awareness and educate communities about the impacts of different types of waste on environmental health and well-being could play a crucial role in mitigating environmental pollution.

4.3.11 Water storage

On different places where the water was kept for use after being fetched from the different sources, 50.6% the respondents indicated that they stored their water openly

(without covering) therefore posing great risks of contamination. Others (24.2%) stored their water in protected areas whilst 25.2% did not store water at all as in Table 4.15.

Table 4.15:

Water storage(N=322)

Water storage	Frequency(n)	Percentage(%)
Open environment	163	50.6
Protected environment	78	24.2
Not applicable	81	25.2

Source: Researcher, (2023)

The findings in Table 4.15 showed that, a significant portion of farmers (about 50.6%) were storing their water openly, without any form of covering. This practice poses a considerable risk of contamination, as open water is vulnerable to various pollutants such as dirt, debris, and even biological contaminants like bacteria and algae. Contaminated water can lead to health issues for both humans and animals, as well as potentially affecting crop growth and livestock health.

On the other hand, a smaller but still notable Percentage of farmers (about 33.8%) were storing their water in protected areas. This approach is much safer as it helps shield the water from external contaminants, reducing the risk of pollution and ensuring a cleaner water supply for agricultural use. Protected storage can involve using covered tanks, barrels, or other containers that prevent outside elements from entering the water.

Surprisingly, a portion of respondents (23.4%) reported not storing water at all. This could be due to various reasons, such as relying on continuous access to fresh water sources or perhaps employing immediate usage of collected water without the need for storage. However, depending solely on real-time access to water sources may not always be reliable, especially in regions where water availability fluctuates seasonally or due to

other factors like droughts or contamination events. According to Liu *et al.* (2018) the findings suggested a need for greater awareness and education regarding proper water storage practices among farmers. Encouraging the adoption of protected storage methods could help mitigate the risks of water contamination, ultimately promoting safer agricultural practices and safeguarding both human health and crop productivity.

The study further conducted Pearson chi-square test at 95% confidence intervals to explore the association between the presence of fecal coliforms in *C. edulis* twigs and the demographic characteristics of the farmers involved in its cultivation. By examining factors such as age, education level, and hygiene practices, the analysis aims to identify potential links between farmer demographics and microbial contamination levels in khat twigs as shown in Table 4.16 and 4.17.

Table 4.16:

Association between fecal coliforms in Catha edulis (khat) twigs and Demographic characteristics of farmers (n=322)

Factors associated with fecal contamination		Low Contamination	Moderate Contamination	High Contamination	Total	Value	df	Asymp. Sig. (2-sided)
Sex	Male	55(17.1%)	75(23.3%)	125(38.8%)	255 (79.2%)	29.762 ^a	2	.000
	Female	3(.9%)	43(13.4%)	21(6.5%)	67 (20.8%)			
Age	15-35	38(11.8%)	18(5.6%)	29(9.0%)	85 (26.4%)	60.671 ^a	4	.000
	35-50	12(3.7%)	66(20.5%)	62(19.3%)	140 (43.5%)			
	50 and Above	8(2.5%)	34(10.6%)	55(17.1%)	97 (30.1%)			
Sanitation Facilities	Yes	46(14.3%)	52(16.1%)	104(32.3%)	202 (62.7%)	28.914 ^a	2	.000
	No	12(3.7%)	66(20.5%)	42(13.0%)	120 (37.3%)			
Type of sanitation facility	Public	22(6.8%)	9(2.8%)	11(3.4%)	42 (13.0%)	47.001 ^a	4	.000
	Private	8(2.5%)	38(11.8%)	26(8.1%)	72 (22.4%)			
	Open/Sharing	28(8.7%)	71(22.0%)	109(33.9%)	208 (64.6%)			
Level of Education	Primary	41(12.7%)	51(15.8%)	86(26.7%)	178 (55.3%)	27.052 ^a	6	.000
	Secondary	13(4.0%)	65(20.2%)	46(14.3%)	124 (38.5%)			
	Tertiary	3(.9%)	1(.3%)	11(3.4%)	15 (4.7%)			
	Did not Attend Any	1(.3%)	1(.3%)	3(.9%)	5 (1.6%)			
Irrigate khat plant	Yes	33(10.2%)	55(17.1%)	73(22.7%)	161 (50.0%)	1.646 ^a	2	0.439
	No	25(7.8%)	63(19.6%)	73(22.7%)	161 (50.0%)			
Type of water used	River	48(14.9%)	52(16.1%)	63(19.6%)	163 (50.6%)	84.304 ^a	4	.000
	Borehole	3(.9%)	42(13.0%)	8(2.5%)	53 (16.5%)			
	Wells	7(2.2%)	24(7.5%)	75(23.3%)	106 (32.9%)			
Farmers feeling	Good	14(4.3%)	1(.3%)	4(1.2%)	19 (5.9%)	44.339 ^a	4	.000
	Ok	43(13.4%)	115(35.7%)	136(42.2%)	294 (91.3%)			
	Bad	1(.3%)	2(.6%)	6(1.9%)	9(2.8%)			
Total	58(18.0%)	118(36.6%)	146(45.3%)	322(100.0%)				

Source: Researcher, (2023}

Table 4.17*Association between fecal coliforms in Catha edulis (khat) twigs and Environmental and Water-Related Factors (N=322)*

Factors associated with fecal contamination		Low Contamination	Moderate Contamination	High Contamination	Total	Value	df	Asymp. Sig. (2-sided)
Kind of waste	Human feaces	21(6.5%)	0(0.0%)	1(.3%)	22 (6.8%)	97.690 ^a	6	.000
	Stagnation of waste	15(4.7%)	56(17.4%)	64(19.9%)	135 (41.9%)			
	Animal feaces	22(6.8%)	61(18.9%)	78(24.2%)	161 (50.0%)			
	All of these	0(0.0%)	1(.3%)	3(.9%)	4 (1.2%)			
Water source	Municipal taps	33(10.2%)	23(7.1%)	25(7.8%)	81 (25.2%)	40.124 ^a	4	.000
	Well	25(7.8%)	90(28.0%)	111(34.5%)	226 (70.2%)			
	Water vendors	0(0.0%)	5(1.6%)	10(3.1%)	15 (4.7%)			
Water storage environment	Open Environment	43(13.4%)	45(14.0%)	75(23.3%)	163 (50.6%)	42.843 ^a	4	.000
	Protected environment	8(2.5%)	49(15.2%)	21(6.5%)	78 (24.2%)			
	Not applicable	7(2.2%)	24(7.5%)	50(15.5%)	81 (25.2%)			
Total	58(18.0%)	118(36.6%)	146(45.3%)	322(100.0%)				

Source: (Researcher, 2023)

The study findings in Table 4.16 revealed that there was a statistically significant association between assessment of fecal coliforms in *C. edulis* twigs and sex $\chi^2 (2, N = 322) = 29.762, p = .000$). This P-value falling below the conventional threshold of .05 suggests a significant association between the two variables. Despite the statistical significance, it is essential to recognize that correlation does not imply causation. These findings show the importance of considering sex as a factor in studies involving fecal coliform assessment in khat twigs. It also disagrees with the study of Syomiti *et al.* (2022) who indicated that there is no significant association between assessment of fecal coliforms in *C. edulis* twigs and sex. Moreover, the study of Walker (2018) noted that there is neither significant association between assessment of fecal coliforms in *Catha edulis* (khat) twigs and sanitation facility.

The study findings also reveal that there was a statistically significant association between assessment of fecal coliforms in *C. edulis* twigs and age $\chi^2 (4, N = 322) = 60.671, p = .000$. The results revealed a statistically significant association, as indicated by a Chi-square value of 60.671 and a corresponding p-value of 0.000 (or $p < 0.001$). This implies a very low probability that the observed association between fecal coliform assessment and age occurred by chance alone. In essence, there appears to be a meaningful relationship between these variables, warranting further investigation and consideration. The findings disagree with the study done by Zerbe (2022) that stated that there is not an association between assessment of fecal coliforms in *Catha edulis* (khat) twigs and age.

Further, the study findings revealed that assessment of fecal coliforms in *C. edulis* twigs is statistically significantly associated with type of sanitation facility $\chi^2 (4, N = 322) = 47.001, p = .000$, this suggests that the choice of sanitation facility directly impacts the levels of fecal coliform contamination observed in khat twigs. Thus, variations in

sanitation practices may serve as crucial determinants of microbial contamination, highlighting the importance of adequate sanitation infrastructure in mitigating contamination risks within khat production and consumption contexts. This finding resonates with research conducted by Mensah and Enu-Kwesi (2023), which emphasized the impact of sanitation facilities on environmental contamination.

The finding on level of education, $\chi^2 (6, N = 322) = 27.052, p = .000$, suggests that there is a meaningful relationship between the level of education and the degree of fecal coliform contamination observed in khat twigs. Individuals with different levels of education may have varying knowledge, attitudes, and practices regarding hygiene, agricultural techniques, and food safety measures. Consequently, these factors could influence their exposure to and management of fecal coliform contamination in khat cultivation and consumption. Sapbamrer and Thammachai (2020) alluded that higher levels of education among farmers are associated with better hygiene practices.

The findings on irrigate khat plant $\chi^2 (2, N = 322) = 1.646, p = .439$, suggests that the method of irrigation employed in khat cultivation does not play a pivotal role in determining the extent of fecal coliform contamination present in harvested twigs. Different irrigation techniques, including the quality and source of water used, Frequency(n) of irrigation, and irrigation management practices, are likely contributing factors to this association. The identification of such a relationship emphasizes the importance of implementing proper agricultural practices to mitigate contamination risks in khat production. Alegbeleye and Sant'Ana (2020) emphasized the importance of using clean irrigation water to minimize the risk of fecal coliform contamination in crops.

The findings on type of water used $\chi^2 (4, N = 322) = 84.304, p = .000$, suggests that the choice of water source for irrigation and other agricultural practices influences the levels of fecal coliform contamination found in khat twigs. Different types of water sources,

such as surface water, groundwater, or treated water, may vary in their susceptibility to fecal contamination, depending on factors such as proximity to human or animal waste sources, water treatment methods, and environmental conditions. Banach and Van Der Fels-Klerx (2020) stated that using untreated or contaminated water for irrigation can introduce fecal coliforms into crops,

The association on farmers feeling $\chi^2(4, N = 322) = 44.669, p = .000$ suggests that farmers' emotional states play a meaningful role in shaping their experiences and practices within khat cultivation. It implies that factors such as job satisfaction, stress levels, and overall emotional well-being significantly influence how farmers engage with their work and manage their farms. Positive emotions may correlate with effective farming practices, while negative emotions could potentially hinder productivity and decision-making processes. The findings concur with McGunnigle *et al.*, (2023) that addressing farmers' emotional needs and fostering a supportive environment, policymakers, agricultural agencies, and community stakeholders can contribute to the resilience and sustainability of khat cultivation practices.

As shown as in Table 4.17, the findings on the association on the kind of waste $\chi^2(6, N = 322) = 97.690, p = .000$, suggest that the nature of waste generated and managed within these agricultural settings plays a pivotal role in influencing contamination levels and environmental health outcomes. Different categories of waste, such as organic, inorganic, liquid, or solid waste, may pose varying risks of contamination, highlighting the importance of tailored waste management strategies to address specific challenges in khat cultivation regions. Zacharia *et al.* (2019) highlighted the importance of proper waste disposal methods to prevent fecal coliform,

The findings on the association of water source $\chi^2(4, N = 322) = 40.124, p = .000$ suggests that the choice of water source for irrigation and other agricultural activities

significantly impacts the levels of fecal coliform contamination observed in khat cultivation. Different water sources, such as rivers, wells, or untreated sources, vary in their susceptibility to contamination, posing varying risks to crop safety and human health. Jofre *et al.* (2021) concurred by stating that there is need for regular monitoring and treatment of water sources to mitigate the risk of fecal coliform.

The findings on the water storage $\chi^2(4, N = 322) = 42.843, p = .000$ suggests that how water is stored significantly impacts contamination levels in khat cultivation. Different storage methods, such as storing water in covered containers versus open reservoirs, can influence the risk of microbial contamination, with improper storage practices potentially compromising water quality and posing risks to agricultural productivity and public health. This agrees with the study done by Holcomb *et al.* (2020) that noted the importance of proper water storage hygiene to prevent fecal coliform contamination.

4.4. Effectiveness of Commonly Used Antimicrobial Drugs of Bacterial Isolates From Khat Twigs

To isolate the single colony of *E.coli* to be used in antibiotic susceptibility test, one loop of the pink/red colonies enumerated were suspended in 1ml of sterile distilled water and plated on selective media (EMB Levine agar, Liofilchem, Roseto, Italy) and the isolate was identified according to its appearance (green metallic sheen).

Table 4.18:

Sensitivity Testing

Type of antibiotic tested	Resistant %	Intermediate %	Susceptible %	Total %
Ceftriaxone	0	0	100.0	100.0
Ampicilin	20.3	21.4	58.3	100.0
Doxyline	9.1	12.3	78.6	100.0

Amoxyline	23.9	15.2	60.9	100.0
Azitromycine	9.8	10.9	79.3	100.0

Source: Researcher, (2023)

The Table 4.18 shows the effectiveness of various commonly used antibiotics on bacterial isolates from khat twigs. The study findings revealed that Cefriaxone antibiotic is the most effective against *E. coli* found on khat twigs with 100% effectiveness. Ampicillin, Amoxyline, Azithromycin antibiotics showed moderate effectiveness, with around 9-24% of the isolates being resistant. Doxycycline antibiotics appears to be the second most effective antibiotic against the bacteria tested.

CHAPTER FIVE: CONCLUSION, RECOMMENDATIONS AND PUBLICATION

5.0 Introduction

This chapter presents the discussion of the study results, conclusions based on the study results and recommendations.

5.1 Summary

5.1.1 Rate of fecal coliforms contamination of *C. edulis* twigs at different stages of value chain

The study sought to assess the rate of fecal coliforms contamination of *C. edulis* twigs at different stages of value chain. The average fecal coliform contamination levels were highest for handlers and lowest for customers. The variability in contamination levels is highest for farmers and lowest for customers, attributed to factors like agricultural practices and handling methods. Minimum and maximum contamination values were similar across all stages, indicating common sources of contamination throughout the supply chain. Contamination levels varied across different stages of the value chain, reflecting differences in hygiene practices and environmental exposures.

From Post-hoc Tests (Tukey's HSD) it was revealed that farmers had significantly lower contamination than handlers, vendors, and customers. There was no significant difference between farmers and vendors, or between handlers and vendors. Contamination increased along the value chain, with the highest levels in customers, emphasizing the need for improved hygiene practices at all stages.

The study findings showed that handlers recorded the highest average contamination levels, whereas customers had the lowest a difference that is statistically significant. It implies that contamination tends to occur more frequently at the earlier stages of food production and handling, which is not entirely surprising given the various points of

contact and potential for mishandling during these stages. Handlers operated under varying levels of hygiene protocols and environmental conditions, leading to greater fluctuations in contamination levels. On the other hand, customers, being the end consumers, are more removed from the direct handling and production processes, hence experiencing lower variability. The study findings were in line with Okpala and Korzeniowska (2023) that variability of faecal coliform contamination levels underscores the importance of implementing consistent and stringent hygiene measures, especially at the primary stages of food production. It also emphasizes the need for effective monitoring and regulation throughout the food supply chain to ensure food safety standards are maintained. Additionally, educating and training handlers and farmers on proper hygiene practices can help mitigate contamination risks and ultimately safeguard public health.

These results concur with a study done by Gezie in the year 2019 where he attributed the higher variability among farmers to factors such as agricultural practices, environmental conditions, and handling methods. The minimum and maximum values for fecal coliform contamination showed similar patterns across all stages, with the lowest minimum value being 3.66 for farmers and the highest maximum value being 4.57 for handlers. This aligns with the study by Yang *et al.* (2022) that noted that the similarity in minimum and maximum contamination values across different stages of the distribution chain may reflect common sources of contamination and similar environmental exposures throughout the supply chain.

The mean levels of fecal coliform contamination are likely to vary significantly across the various stages of the value chain, including farmers, handlers, vendors, and customers. This suggests that different stages of the value chain may contribute differently to fecal coliform contamination, highlighting potential areas for intervention

or improvement in food safety practices. This is in line with the study done by Ahmedsham *et al.* (2018) that indicated that microbial contamination levels vary significantly throughout the distribution chain, indicating potential differences in hygiene practices, environmental exposures, and handling methods at each stage.

The post-hoc tests (Tukey's HSD) for pairwise comparisons findings revealed that farmers have significantly lower fecal coliform contamination than handlers, vendors, and customers. This agrees with the study done by Olivas. (2018) that found that farmers consistently exhibited lower levels of fecal coliform contamination in comparison to handlers, vendors, and customers. Handlers have significantly more contamination than customers. A study by Nthiga (2022) supports the finding that handlers have significantly higher contamination levels compared to customers in the context of *Catha edulis* (khat) distribution. There was no significant difference between farmers and vendors, or between handlers and vendors. The study by Roth *et al.* (2018) found that both farmers and vendors exhibited similar levels of fecal coliform contamination, suggesting no significant difference between these groups

These results suggested that fecal coliform contamination increased as Khat twigs moved through the value chain, with the highest levels found in customers. This highlights the need for improved hygiene practices at all stages, especially for handlers and vendors. This study aligns with the finding by Zerbe (2022) that noted that fecal coliform contamination increases as *C. edulis* twigs move through the value chain, emphasizing the importance of implementing enhanced hygiene protocols and quality control measures at all stages of the distribution process.

5.1.2 Factors associated with the fecal coliforms contamination of catha edulis (khat) twigs at different stages of value chain

The study findings revealed that there was a statistically significant association between assessment of fecal coliforms in *C. edulis* twigs and gender. These findings showed the importance of considering gender as a factor in studies involving fecal coliform assessment in khat twigs. It also disagrees with the study of Syomiti *et al.* (2022) who indicated that there is no significant association between assessment of fecal coliforms in *C. edulis* twigs and sex. Moreover, the study of Walker (2018) noted that there is neither significant association between assessment of fecal coliforms in *C. edulis* twigs and sanitation facility. There was a statistically significant association between assessment of fecal coliforms in *C. edulis* twigs and age. The findings disagree with the study done by Zerbe (2022) that stated that there is not an association between assessment of fecal coliforms in *C. edulis* twigs and age.

Assessment of fecal coliforms in *C. edulis* twigs was statistically significant associated with type of sanitation facility. This suggests that the choice of sanitation facility directly impacts the levels of fecal coliform contamination observed in khat twigs. Thus, variations in sanitation practices may serve as crucial determinants of microbial contamination, highlighting the importance of adequate sanitation infrastructure in mitigating contamination risks within khat production and consumption contexts. This finding resonates with research conducted by Mensah and Enu-Kwesi (2023), which emphasized the impact of sanitation facilities on environmental contamination.

There was a meaningful relationship between the level of education and the degree of fecal coliform contamination observed in khat twigs. Individuals with different levels of education had varying knowledge, attitudes, and practices regarding hygiene, agricultural

techniques, and food safety measures. Sapbamrer and Thammachai (2020) alluded that higher levels of education among farmers are associated with better hygiene practices.

Different irrigation techniques, including the quality and source of water used, Frequency(n) of irrigation, and irrigation management practices, were likely contributing factors to this association. The identification of such a relationship emphasizes the importance of implementing proper agricultural practices to mitigate contamination risks in khat production. Alegbeleye and Sant'Ana (2020) emphasized the importance of using clean irrigation water to minimize the risk of fecal coliform contamination in crops.

Different types of water sources, such as surface water, groundwater, or treated water, may vary in their susceptibility to fecal contamination, depending on factors such as proximity to human or animal waste sources, water treatment methods, and environmental conditions. Banach and Van Der Fels-Klerx (2020) stated that using untreated or contaminated water for irrigation can introduce fecal coliforms into crops, Farmers' emotional states played a meaningful role in shaping their experiences and practices within khat cultivation. It implies that factors such as job satisfaction, stress levels, and overall emotional well-being significantly influence how farmers engage with their work and manage their farms. Positive emotions may correlate with effective farming practices, while negative emotions could potentially hinder productivity and decision-making processes. The findings concur with McGunnigle *et al.*, (2023) that addressing farmers' emotional needs and fostering a supportive environment, policymakers, agricultural agencies, and community stakeholders can contribute to the resilience and sustainability of khat cultivation practices.

Different categories of waste, such as organic, inorganic, liquid, or solid waste, may pose varying risks of contamination, highlighting the importance of tailored waste management strategies to address specific challenges in khat cultivation regions.

Zacharia *et al.* (2019) highlighted the importance of proper waste disposal methods to prevent fecal coliform,

Different water sources, such as rivers, wells, or untreated sources, varied in their susceptibility to contamination, posing varying risks to crop safety and human health.

Jofre *et al.* (2021) concurred by stating that there is need for regular monitoring and treatment of water sources to mitigate the risk of fecal coliform.

Different storage methods, such as storing water in covered containers versus open reservoirs, could influence the risk of microbial contamination, with improper storage practices potentially compromising water quality and posing risks to agricultural productivity and public health. This agrees with the study done by Holcomb *et al.* (2020) that noted the importance of proper water storage hygiene to prevent fecal coliform contamination.

5.1.3 Effectiveness of commonly used antimicrobial drugs on bacterial isolates from khat twigs

The study findings revealed that Ceftriaxone antibiotic was the most effective against *E. coli* found on khat twigs with 100% effectiveness. Ampicillin, Amoxyline, Azithromycin antibiotics showed moderate effectiveness, with around 9-24% of the isolates being resistant. Doxycycline antibiotics appears to be the second most effective antibiotic against the bacteria tested.

5.2 Conclusion

The study concluded that fecal coliform contamination levels vary across different stages of the supply chain, with the highest levels found in handlers and the lowest in farmers.

The variability in contamination levels was highest for farmers and lowest for customers, likely due to factors such as agricultural practices and handling methods. Post-hoc tests showed that farmers had significantly lower contamination levels compared to handlers,

vendors, and customers, with no significant difference between farmers and vendors, or between handlers and vendors.

Further, younger and male farmers were more likely to engage in khat cultivation compared to older and female farmers. Proper sanitation facilities were associated with lower fecal coliform contamination in khat crops. Higher levels of education among farmers led to better hygiene practices and reduced contamination risks. Proper irrigation practices and the use of clean water sources were crucial in preventing fecal coliform contamination in khat plants. Farmers' attitudes towards hygiene practices and waste management impacted the likelihood of contamination. The presence of fecal coliforms in khat twigs was significantly associated with the type of sanitation facility, level of education, irrigation practices, water sources, farmers' feelings, kind of waste, water source, and water storage.

The study furthermore concluded that there were varying levels of effectiveness of common antimicrobial drugs against bacterial isolates from khat twigs. While some antibiotics like Doxycycline demonstrated good efficacy across different groups.

5.3 Recommendations and Suggestions for Future Studies

Improve hygiene practices at all stages of the khat value chain: This is particularly important for handlers and customers, as they had the highest contamination levels. This could involve training on proper handwashing, sanitation of surfaces and equipment, and proper storage of khat twigs.

While farmers have lower contamination levels compared to other stages, there's still room for improvement. Educational programs could target best practices in irrigation, sanitation facilities, and waste management. Valuable sanitary facilities must be available to minimize the risks of fecal contamination. The adoption of protective irrigation techniques together with clean water resource selection should be supported for

farmers. The khat farming operation must follow mandatory hygiene guidelines which will protect product safety together with production quality consistency. Controlling khat acquisition must start by consumers checking which vendor stands for maintaining hygiene practices. Proper khat leaf preparation for consumption includes cleaning the twigs through thorough washing because this reduces the chances of eating fecal coliforms. Future studies should analyze the mechanisms and direct relationships which exist between fecal coliforms in khat twigs and the genders of those who handle or produce khat products.

Continuous monitoring of antimicrobial resistance patterns in bacterial isolates from khat twigs is crucial to guide appropriate therapy. Promoting awareness among farmers, handlers, vendors, and customers about prudent antibiotic use can help combat resistance. Exploring alternative antimicrobial agents or combinations that show higher efficacy against these bacterial isolates could be beneficial.

Conduct longitudinal studies to track the impact of interventions aimed at reducing fecal coliform contamination in khat cultivation. This would provide more robust evidence on the effectiveness of various strategies.

Investigate the effectiveness of different types of sanitation facilities (e.g., pit latrines vs. composting toilets) in reducing fecal coliform contamination in khat crops.

Conducting more studies to understand the mechanisms behind antibiotic resistance in these bacterial isolates for better treatment strategies.

5.4 Publication

Gilbert Keter, Dorothy Kagendo , Josephine Mutembei, Assessment of fecal coliforms in *Catha edulis* (Khat) leaves along the value chain in Igembe South Sub-County, Meru, Kenya. African , *Journal of Science, Technology and Social Sciences*, 4(2), 9-16.” <https://doi.org/10.58506/ajstss.v3i2.248>

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APPENDICES

Appendix A: Journal Article Publication

AFRICAN JOURNAL OF SCIENCE, TECHNOLOGY AND SOCIAL SCIENCES. 3(2) 2024, SS



AFRICAN JOURNAL OF SCIENCE,
TECNOLOGY AND SOCIAL SCIENCES

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A publication of Meru University of Science and Technology

Assessment of Fecal Coliforms in *Catha edulis* (Khat) Leaves Along the Value Chain in Igembe South Sub-County, Meru, Kenya.

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ARTICLE INFO

Keywords

Fecal coliforms contamination

Assessment

Sanitation practices and Value chain.

ABSTRACT

Catha edulis leaves have been found to contain high levels of fecal coliforms, exceeding recommended limits for safe consumption, therefore posing a high risk of spreading bacterial diseases. The presence of fecal coliforms in these leaves is a concern for consumers and those involved in their production and transportation. Therefore, this study assessed the fecal coliform contamination rate of *Catha edulis* (Khat) leaves at different stages of the value chain in Igembe South Sub-County, Meru, Kenya. This study was conducted and it involved 328 respondents from 25 locations

within the Sub-County, and it adopted a cross-sectional descriptive study design involving one-time sampling of Khat leaves during farming, handling, vending, and consumption. Khat leaves were collected in aerated bags, processed, and evaluated for the presence of fecal coliforms at the Meru University of Science and Technology Laboratory. Data collected were analyzed using descriptive statistics such as mean, standard deviation, minimum, and maximum and inferential statistics such as Tukey HSD with the aid of Statistical Package of Social Sciences (SPSS) version 23.0. Study findings from Tukey HSD revealed that handlers had significantly higher fecal coliform contamination with mean difference=0.1162; $p=.000$ than vendors (mean difference=0.0994; $p=.002$), customers (mean difference=0.1096; $p=.001$) and farmers having the lowest with mean difference=0.0169; $p=.000$. The study concluded that handling was the most contributing factor to fecal coliform contamination. The study recommends the need to reduce fecal coliform contamination among handlers as well as vendors, customers and farmers by improving sanitation hygiene practices such as handwashing, proper use of sanitation facilities, sanitation of surfaces and equipment and proper storage of khat leaves.

Introduction

Fecal coliforms are a group of bacteria found in the intestines of mammals, including humans, and are often used as indicators of microbial contamination to depict that the fecal matter is not safely managed from the point of generation, containment, transportation, treatment and disposal or end use (Tomina-ga, 2019). Fecal coliforms are also involved in food spoilage and can cause illness in both humans and an-

imals. Coliform counts are generally used as an indicator of possible fecal contamination and reflect the hygiene standards adopted in the food's preparation (Yuan et al. 2019). Globally, there has been concern regarding the levels of fecal coliforms found in *Catha edulis* leaves, a popular stimulant in many cultures.

Regionally, *Catha edulis* is commonly chewed in many African countries due to its numerous pharmacolog-

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Appendix B: Participant Consent Form

Title: Assessment of fecal coliforms in *Catha edulis* (Khat) twigs along the value chain in Igembe South Sub-County, Meru, Kenya.

Investigator: Keter Gilbert Kiplagat

Purpose of the study: I am a master's student at Meru University of Science and Technology and this study is part of my completion of degree. The study title is **assessment of fecal Assessment of Fecal Coliforms in Catha edulis (Khat) Twigs Along the Value Chain in Igembe South Sub-County, Meru, Kenya.** This study helped the principal investigators gain skills in fecal coliforms as indicators of microbial contamination of *Catha edulis* (khat) twigs at different stages of value chain on research methods, therefore the data to be gathered would be analyzed and published.

Procedure; during this study, you are asked to complete a number of brief questions concerning fecal coliforms as indicators of microbial contamination of *Catha edulis* (khat) twigs at different stages of value chain. Your participation will require approximately 10 minutes of your time.

Confidentiality; All records of participation would be kept strictly confidential, such that only I and my team will have access to the information. Data would be stored in a locked filing in an office which would be destroyed by shredding at the end of the project, electronic files will also be deleted at that time. The results from the study would be reported in a written research report and oral report during presentation. Information about the project did not be made to the public in any way that identifies individual participants.

Risks and benefits; there are no risks during the research and there may be no personal benefits from your participation but the knowledge received may be of educational value to the researcher with regards to methods of conducting the research.

Participation; Your participation is completely voluntary. You may withdraw at any time for any reason without explanation and penalty. You may choose not to answer any question for any reason.

If you have any questions concerning the study or your treatment you could contact...

Keter Gilbert Kiplagat: Tel +254 725 644337 / gilbertketer90@gmail.com

Agreement I have read the above form, understood the information read, understand
that I can ask questions and understand the procedure. I voluntarily agree to participate in this study.

Participants initials.

Date

Appendix C : Questionnaire for Miraa Farmers

[N.B: Investigator will pose as potential consumer without antagonizing farmer.]

Questionnaire Number.....

(QUESTIONNAIRE FOR FARMERS)

Information requested herein is for study purpose only and would be taken in

1. Residence/location
Sex. Male Female
2. Age a.15-35 b. 35- 50 c. 50 and above
3. Are there any sanitation facilities within the farm?
a. Yes No
4. If yes, what type are they?
a. Public b. Personal c. Open/sharing toilet
5. What is your level of education?
a. Primary b. Secondary c. Tertiary D. Did not attend any
6. Do you irrigate the Khat Plant
a. Yes No
b. If yes, what type of water do you use
i. River ii. Borehole iii. wells iv Treated waste water

Tick Where Applicable (Principle investigator will tick where applicable as he buys the twigs from vendor)

7. How do you feel that the cleanness in your local environment?
a) Good b) Ok c) Bad
8. What kind of waste found in environment?
a) Human feces b) Stagnation of waste c) Animal feces d) All of these
9. Where do you get water?
a) Municipal taps b) well c) Water vendors
10. Where do you keep water?
a) Open environment b) Protected environment c) Not applicable

This section to be filled in the lab

10. Counting the number of colonies on the culture plate

Sample id	Area location	Number of coliforms	Number of coliforms
	where the sample	counted on original	counted on the plate

	is collected	sample	of serial dilution 10^{-1}

Appendix D: Questionnaire for Miraa Handlers

[N.B, Investigator will pose as potential consumer without antagonizing farmer.]

Questionnaire Number.....

(QUESTIONNAIRE FOR HANDLERS)

Information requested herein is for study purpose only and would be taken in

1. Residence/location
Sex. Male Female
 2. Age
 3. Are there any sanitation facility within the places where Khat Twigs is being handled?
a. Yes No
 4. If yes, what type are they?
a. Public b. Personal c. Open/sharing toilet
 5. What is your level of education?
a. Primary b. Secondary c. Tertiary D. Did not attend any
 6. Do you wash your hands before handling the Khat Twigs?
a. Yes No
 7. If yes, where do you get water?
a) Municipal taps b) well c) Water vendors
 8. Do you experience water shortage some times?
a. Yes b. No
- Tick Where Applicable (Principle investigator will tick where applicable as he buys the twigs from vendor)
9. How do you feel that the cleanness in your local environment?
a) Good b) Ok c) Bad
 10. What kind of waste found in environment?
a) Human feces b) Stagnation of waste c) Animal feces d) All of these
 11. Where do you keep water?
a) Open environment b) Protected environment c) Not applicable

This section to be filled in the lab

10. Counting the number of colonies on the culture plate

Sample id	Area location where the sample is	Number coliforms counted	of	Number coliforms counted	of
-----------	--------------------------------------	-----------------------------	----	-----------------------------	----

	collected	on original sample	on the plate of serial dilution 10^{-1}

Appendix E: Questionnaire for Miraa Vendors

[N.B, Investigator will pose as potential consumer without antagonizing farmer.]

Questionnaire Number.....

(QUESTIONNAIRE FOR VENDORS)

Information requested herein is for study purpose only and would be taken in

1. Residence/location
Sex. Male Female
 2. Age
 3. Is there any sanitation facility within the places where Khat Twigs is being handled?
a. Yes No
 4. If yes, what type are they?
a. Public b. Personal c. Open/sharing toilet
 5. What is your level of education?
a. Primary b. Secondary c. Tertiary D. Did not attend any
 6. Do you wash your hands before handling the Khat Twigs?
a. Yes No
 7. If yes, where do you get water?
a) Municipal taps b) well c) Water vendors
 8. Do you experience water shortage some times?
a. Yes b. No
- Tick Where Applicable (Principle investigator will tick where applicable as he buys the twigs from vendor)
9. How do you feel that the cleanness in your local environment?
a) Good b) Ok c) Bad
 10. What kind of waste found in environment?
a) Human feces b) Stagnation of waste c) Animal feces d) All of these
 11. Where do you keep water?
a) Open environment b) Protected environment c) Not applicable

This section to be filled in the lab

10. Counting the number of colonies on the culture plate

Sample id	Area location where the sample is	Number of coliforms counted	Number of coliforms counted

	collected	on original sample	on the plate of serial dilution 10^{-1}

Appendix F: Questionnaire for Miraa Customers

[N.B, Investigator will pose as potential consumer without antagonizing farmer.]

Questionnaire Number.....

(QUESTIONNAIRE FOR CUSTOMER)

Information requested herein is for study purpose only and would be taken in

1. Residence/location
Sex. Male Female
 2. Age
 3. Are there any sanitation facilities within the places where Khat Twigs is being handled?
a. Yes No
 4. If yes, what type are they?
a. Public b. Personal c. Open/sharing toilet
 5. What is your level of education?
a. Primary b. Secondary c. Tertiary D. Did not attend any
 6. Do you wash your hands before handing the Khat Twigs?
a. Yes No
 7. If yes, where do you get water?
a) Municipal taps b) well c) Water vendors
 8. Do you experience water shortage some times?
a. Yes b. No
- Tick Where Applicable (Principle investigator will tick where applicable as he buys the twigs from vendor)
9. How do you feel that the cleanness in your local environment?
a) Good b) Ok c) Bad
 10. What kind of waste found in environment?
a) Human feces b) Stagnation of waste c) Animal feces d) All of these
 11. Where do you keep water?
a) Open environment b) Protected environment c) Not applicable

This section to be filled in the lab

10. Counting the number of colonies on the culture plate

Sample id	Area location where the sample is	Number of coliforms counted	Number of coliforms counted

	collected	on original sample	on the plate of serial dilution 10^{-1}

Appendix G: Antimicrobial Susceptibility Patterns.

Type of antibiotic tested		Resistant	Intermediate	Susceptible	Total
Cefriaxone	F				
	%				
Ampicilin	F				
	%				
Doxyline	F				
	%				
Amoxyline	F				
	%				
Azitromycine	F				
	%				

Appendix H: Introductory Letter



MERU UNIVERSITY OF SCIENCE & TECHNOLOGY

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SCHOOL OF ENGINEERING AND ARCHITECTURE DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

TO: Whom It may concern

DATE: 21st August, 2023

Dear Sir/Madam,

RE: INTRODUCTORY LETTER FOR GILBERT KIPLAGAT KETER, REG NO. EG407/201430/21

The above-named, is a student in the Department of Civil and Environmental Engineering at Meru University of Science and Technology, pursuing a Master's degree in Sanitation. He has been approved to conduct research on "Assessment of Fecal Coliforms as indicators of Microbial Contamination of Catha Edulis (Khat) Leaves at Different Stages of Value Chain in Igembe South Sub-County, Meru, Kenya" aimed at completing his studies. This is therefore, to request that you grant him any assistance needed to enable him meet the program requirements for his graduation.

Kindly contact us for any further enquiries.

Thank you

A handwritten signature in blue ink, appearing to read "Mirara Simon W.", with a horizontal line underneath.






Mirara simon w.
Chair of Department Civil and Environmental Engineering
Meru University of Science and Technology

Email: CODcivilengineering@must.ac.ke
Smirara@must.ac.ke



MUST IS ISO 9001:2015 CERTIFIED

Appendix I : Research Licence

 REPUBLIC OF KENYA	 NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION
Ref No: 616712	Date of Issue: 11/September/2023
RESEARCH LICENSE	
	
This is to Certify that Mr.. Gilbert Kiplagat Keter of Meru University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Meru on the topic: ASSESSMENT OF FECAL COLIFORMS IN CATHA EDULIS (KHAT) LEAVES ALONG THE VALUE CHAIN IN IGEMBE SOUTH SUB-COUNTY, MERU, KENYA. for the period ending : 11/September/2024.	
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616712 Applicant Identification Number	 Director General NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION
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Appendix J: Plagiarism Report

