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

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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 manage from the point of generation, containment, transportation, treatment and disposal or end use (Tominaga, 2019). Fecal coliforms are also involved in food spoilage and can cause illness in both humans and animals. 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 edu-

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lis leaves, a popular stimulant in many cultures.

Regionally, *Catha edulis* is commonly chewed in many African countries due to its numerous pharmacological effects. However, the widespread consumption of *Catha edulis* leaves 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 *Catha edulis* leaves is crucial in ensuring public health safety.

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). 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). Similarly, khat is highly susceptible to contamination from soil and water during harvest and transportation, which could expose consumers to pathogens such as *Escherichia coli.*

Current use of miraa in Kenya is 3.9%; it also varies by region of residence and gender. Although non-users both in rural and urban areas condemn the practice of chewing khat, the number of people who use this plant is increasing, particularly among the youth. In urban areas, chewing khat is becoming a common leisure activity. Like tobacco products, use of miraa is largely a male dominated affair. The consumption of contaminated khat poses 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).

These are individuals who infrequently undergo medical inspections despite Kenya's health requirements that all food-related handlers undergo regular medical examination to curb possible transmission of disease-causing agents (Kamau et al., 2012). It is against this background that this study was born. Hence, the objective of this study was to assess the rate of fecal coliforms contamination of *Catha edulis* (Khat) leaves at different stages of value chain in Igembe South Sub-County, Meru, Kenya.

Material and Methods

Location of study

The study was carried out in Meru County particularly Igembe South Sub County where Khat plant is mostly grown.

Study Population

The study population included 430 khat farmers, 795 handlers, 318 vendors and 281 users from Igembe South Sub-County, Meru County, Kenya.

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.

Sample Size Determination

This study used Yamane's formula, to determine the sample size of 328 respondents.

Sampling Techniques

Collection of *C. edulis* from study site was done with the help of two research assistant from all the stages of value chain of Khat, an average of 300g of khat leaves per site were obtained and stored in a cool dry aerated bags during transportation. A questionnaire having a number for confidentiality was used for each enrolled participant without antagonizing the participant in an understandable language where necessary. The purpose of the questionnaire was to obtain bio-data (source of *C. edulis* leaves, site of respondents, and public health awareness on infection transmission, source of social amenities such as sewerage, toilet, food and water.

Sampling protocol for Khat leaves

The Produce Sample Collection and Processing Form that has been pre-labeled with a Sample ID was reprieved and date and time of the sampling location recorded. Labeled Whirl-Pak bag was opened by gently pulling out the tabs of the side of the bag without touching the mouth or inside of the bag and 300 grams of Khat leaves placed inside then closed. The whirl-pak bag was placed in the ice chest with ice pack the transported to the lab within 6 hours to be stored at 4°C refrigerator until they are ready to be analyzed.

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. The leaves were then removed from the Whirl- Pak bag and the sample stored at 4°C until when they were ready for processing.

Culturing of fecal coliforms.

The procedure was performed according to Sanipath Assessment tool whereby Serial dilutions (up to 2-fold i.e. 10-1 and 10-2) of the original suspension of coliforms in Whirl park bag was done and 100 micro liters from each dilution factor were spread on Mackonkey agar (selective and differentiating media for fecal coliform bacteria) using the glass spreader and incubated at 37 ºC for 24 hours. In order to identify the present of fecal coliform bacteria there must a growth on the media (petri dishes) which are pink or red in color. These growth (colonies) were counted and the number obtained was used to determine the Colony Forming Units per gram of the sample.

Data analysis

Data collected were analyzed using descriptive statistics such as mean, standard deviation, minimum, and maximum and inferential statistics such as ANOVA with the aid of Statistical Package of Social Sciences (SPSS) version 23.0. Data findings were presented in tables and graphs besides narrative descriptions. The level of statistical significance was considered as $P < 0.05$.

Ethical Considerations

The interviews were conducted with the utmost respect and without judgment. All research activities were performed to ensure the participants were not exposed to embarrassment or exploitation and were informed they could withdraw from the study at any time. Furthermore, the research complied with the regulations regarding ethical research practice. The research proposal was submitted to MIRERC and NACOSTI to check on the research project's aims and design and see if it conforms to the institution's code of conduct. Approval from the local administrative units was also sought before the commencement of data collection within Igembe South Sub county, and the participants signed informed consent before participating in the study.

Results

Out of 328 intended samples of Khat leaves 322 (98.2%) were collected.

Rate of Fecal Coliforms Contamination of Catha edulis (khat) leaves at different stages of value chain

The study sought to assess the rate of fecal coliforms contamination of Catha edulis (khat) leaves 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 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 1. This data suggests an interesting pattern in fecal coliform contamination levels across different groups involved in food handling. The handlers had the highest average contamination levels compared to customers which implies that contamination tends to occur more frequently during handling.

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) 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 maintain food safety standards. 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 (Gezie (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 Xu 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 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 2 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.

Therefore, the conclusion drawn is that 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. 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.

	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
Total	322	4.0235	.18099	3.66	4.57

Table 1: *Descriptive Analysis*

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

Table 2: *ANOVA Results*

(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

Table 3: *Tukey HSD*

Based on observed means.

The error term is Mean Square(Error) = .030.

**. The mean difference is significant at the .05 level.*

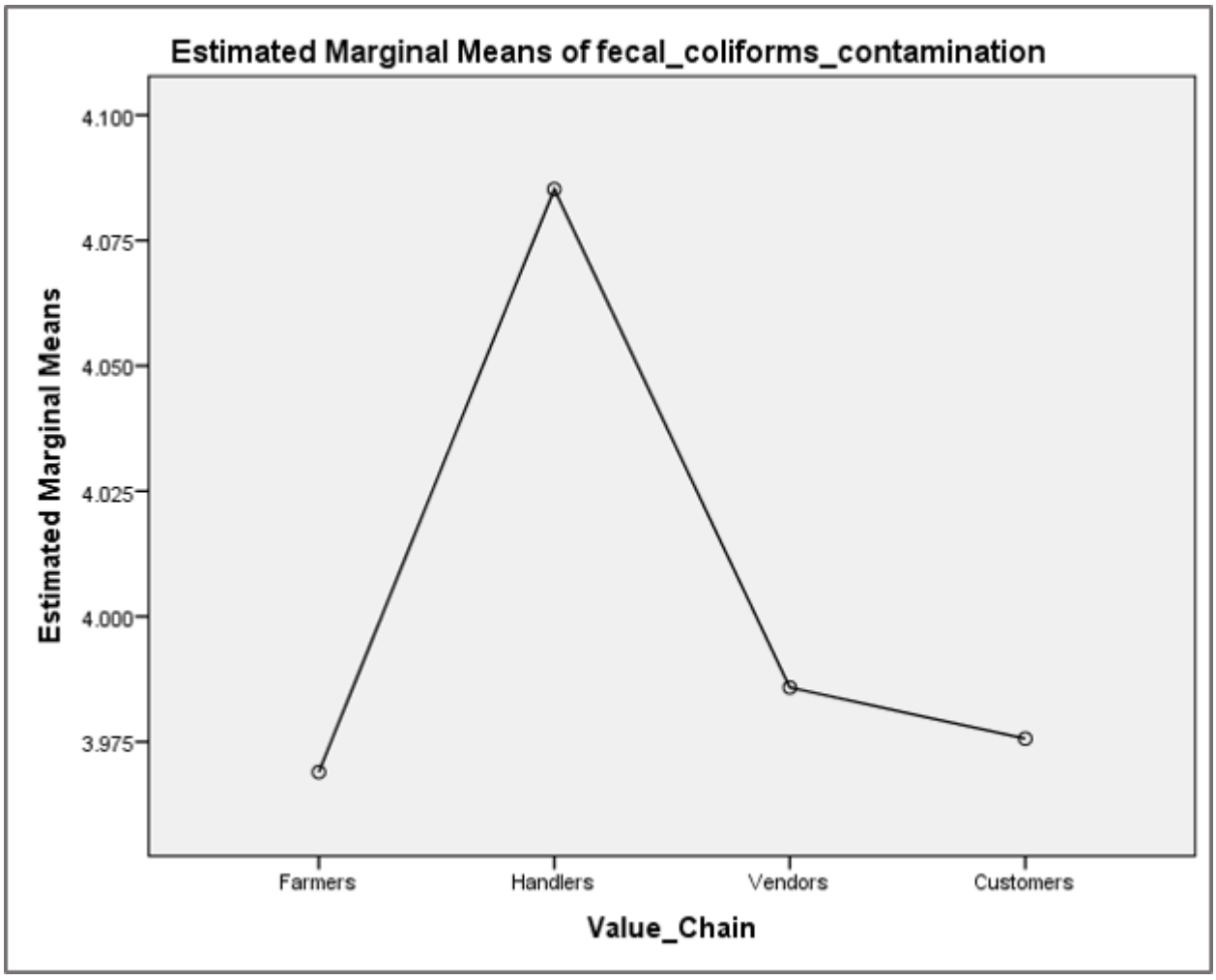

Figure 1: *Estimated marginal means of fecal coliforms contamination*

Farmers have significantly lower fecal coliform contamination than handlers, vendors, and customers (marked with *). 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) 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

All other comparisons with farmers or customers had a p-values exceeding 0.05, meaning there's not enough evidence to conclude a significant difference. These results suggest that fecal coliform contamination increases as Khat leaves 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 increases as *Catha edulis* leaves move through the value chain, emphasizing the importance of implementing enhanced hygiene protocols and quality control measures at all stages of the distribution process.

Conclusions

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 is highest for farmers and lowest for customers, likely due to factors such as ag-

ricultural practices and handling methods. Post-hoc tests showed that farmers have significantly lower contamination levels compared to handlers, vendors, and customers, with no significant difference between farmers and vendors, or between handlers and vendors.

Recommendations

Improve hygiene practices at all stages of the khat value chain: This is particularly important for handlers and customers, as they have the highest contamination levels. This could involve training on proper handwashing, sanitation of surfaces and equipment, and proper storage of khat leaves. While farmers have lower contamination levels compared to other stages, there's still room for improvement.

Suggestions for further study

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.

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