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COMPARATIVE STUDY ON MICROBIAL CONTAMINATION IN LEAFY VEGETABLES GROWN USING DIFFERENT FARMING METHODS

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ABSTRACT

The study was conducted during December 2023 to February 2024 to evaluate the microbial quality of the most commonly consumed leafy vegetables (LVs) in India, i.e., coriander, mint, and fenugreek leaves, grown using conventional, organic, and hydroponic farming methods. The aim was to determine whether organic and hydroponic practices offer safer alternatives to conventional farming. Samples were collected from conventional (local) markets, and organic and hydroponic farms located in Jaipur, India, and analysed for total mesophilic aerobic count (TMAC), total coliform count (TCC), and *Escherichia coli* (*E. coli*) using standard microbiological procedures. Results revealed significant variations in microbial quality across farming systems. Conventionally grown samples exhibited the highest TMAC, with coriander leaves recording 4.55×10^9 CFU g^{-1} (colony forming unit g^{-1}), while TMAC in all three conventionally grown LVs exceeded the FSSAI permissible limit (10^5 CFU g^{-1}). In contrast, organically grown samples showed moderate TMAC values (10^4 –10^5 CFU g^{-1}), whereas hydroponically grown samples consistently exhibited the lowest microbial loads (10^3 CFU g^{-1}). A similar trend was observed for TCC, where conventionally grown coriander leaves showed the highest levels (4.06×10^6 CFU g^{-1}), while organic and hydroponic counterparts had undetectable levels (<1 CFU g^{-1}). The differences among farming methods were statistically significant ($p < 0.05$). Notably, *E. coli* O157 was detected only in conventionally grown coriander leaves from Muhana Mandi (1.05×10^{10} CFU g^{-1}), indicating poor sanitation and possible sewage water contamination. The findings suggest that hydroponic and organic farming practices yield LVs with superior microbial quality compared to conventional methods. Additionally, reduced contamination in hydroponic produce highlighted the potential of controlled environment farming as a safer and sustainable alternative for urban consumers.

(Key words: Conventional, hydroponic, leafy vegetables, microbial contamination, organic)

INTRODUCTION

Microorganisms, or microbes, are microscopic living organisms that include bacteria, viruses, fungi, and protozoa. They are present everywhere and play essential roles in ecosystems, including nutrient recycling, fermentation, maintaining gut health in humans, and the functioning of the biosphere (Crowther *et al.,* 2024). While developed nations regulate microbial safety, India, despite being the second-largest vegetable producer (producing around 200 million metric tons of vegetables annually), faces challenges due to limited infrastructure (Anonymous, 2023a). Among all the vegetables, leafy vegetables (LVs) are an important part of Indian meals containing vitamins such as A, C, K, and folate as well as minerals such as calcium, iron, and magnesium, dietary fiber, and bioactive compounds like antioxidants and phenolics (Bharai, 2024; Sarkar *et al.,* 2023). Due to this extremely diverse nutritional profile, LVs are widely acknowledged as key benefactors to healthy diet (Sarkar *et al.,* 2023).

LVs, though highly nutritious, are prone to microbial contamination due to their moisture, texture, and large surface area, especially when consumed raw (Bharai, 2024). Contamination can occur at any stage from farm to fork, with studies reporting pathogens such as *E. coli, Salmonella, Listeria, Staphylococcus,* and *Klebsiella* in market samples and fresh produce worldwide (Kalpana *et al.,* 2024; Mritunjay and Kumar, 2017). Among the wide variety of LVs, coriander (*Coriandrum sativum*), mint (*Mentha spicata*) and fenugreek (*Trigonella foenum-graecum*) are the most commonly consumed LVs due to their distinctive flavors and significant place in Indian cuisine. A survey in Andhra Pradesh reported 100% consumption of coriander, 99% mint, and 95% fenugreek (Sai Lakshmi *et al.,* 2020). Given their widespread usage and minimal preparation, ensuring the microbial safety of these commonly consumed LVs becomes especially critical in the broader context of food safety in India.

Significant scientific innovations and advancements have been made in the field of agriculture.

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Along with the traditional farming method, i.e., conventional farming, the use of alternative agricultural practices, such as organic and hydroponic farming has seen emerged. Organic farming relies on natural resources (Kashyap and Jain, 2025), while hydroponic farming, involves soilless production, where a nutrient-rich solution is used (Kashyap and Jain, 2024; Pawar *et al.,* 2024). However, evidence on the microbiological quality of LVs from these systems is limited. In this context, the present study was undertaken to assess and compare the microbial load in three commonly consumed LVs purchased from conventional market, organic farm and hydroponic farm.

MATERIALS AND METHODS

Samples of three selected LVs were collected in triplicates from conventional, organic, and hydroponic markets in Jaipur (Rajasthan, India). All samples were collected in laboratory-specialized zipper pouches (Merck Z162930) to prevent contamination. The testing was performed on the same day as sample collection at the IRCLASS Systems and Solutions Pvt Ltd Laboratory, Jaipur, Rajasthan (NABL/ISO 17025 accredited and approved by FSSAI, EIC, and APEDA) from December 2023 to February 2024. All LVs of the same type/variety were analyzed simultaneously and under the same conditions.

The media used in the study were purchased from HiMedia Laboratories (Mumbai, Maharashtra, India) and were prepared as mentioned in their technical data (HiMedia 2024). The samples were prepared in a laminar airflow (Optics Technology, Delhi, India). For total mesophilic aerobic count (TMAC), plate count agar (PCA) (HiMedia M091) medium (previously autoclaved and maintained at 45°C ± 10°C) was poured into the inoculated plates and gently rocked in both clockwise and counter clockwise directions to spread and cool. The plates were packed and incubated at 30ºC for 72 hours (Anonymous, 2012a). Total coliform count (TCC) was determined using violet red bile agar (VRBA) (HiMedia M049) medium (previously autoclaved and maintained at 45°C ± 1°C). Inoculated plates of VRBA with six dilutions were incubated at 37°C for 24 hours (Anonymous, 2012b). At the end of incubation, the plates were removed and colonies were counted using the colony counter (Model No: 363, Electronics, India) and calculated using the following formula (Anonymous, 2012a; Anonymous, 2012b):

$$N = \frac{\sum C}{V[n1 + (0.1n2)] * d}$$

$\sum$C=Sum of colonies on all the dishes retained from two successive dilutions

V=Volume of inoculum applied on each dish (in ml)

n1=Number of dishes retained at first dilution

n2=Number of dishes retained at second dilution

d=Dilution factor corresponding to the first dilution retained

A qualitative test for *Escherichia coli* (*E. coli)* O157 was carried out using the tests mentioned in Anonymous, (1976). A 25 g sample was blended with 200 ml of 0.1% peptone salt solution (PSS) (HiMedia M1748), inoculated into MacConkey broth (HiMedia MH083) and MacConkey agar (HiMedia MH081), and incubated at 37°C overnight. Growth was identified by acid and gas production, and characteristic colonies (Plate 1). The microscopic image of *E. coli* is shown in Plate 2. The microbial results obtained from TMAC, TCC, and *E. coli* O157 analyses were interpreted with reference to acceptable microbiological limits. According to the Food Safety and Standards Authority of India (FSSAI), ready-to-eat fresh vegetables should not exceed 10^5 CFU g^{-1} (colony forming unit g^{-1}) for TMAC and 10^2 CFU g^{-1} for TCC. Additionally, the presence of *E. coli* O157 was considered unacceptable in 25 g of any sample (Anonymous, 2023b).

The results obtained for conventional, organic, and hydroponic LVs were compared using one way analysis of variance (ANOVA) test. All tests were conducted using SPSS (Statistical Package for the Social Sciences) software version 20, with a significance level of 0.05.

RESULTS AND DISCUSSION

The microbial analysis of coriander, fenugreek, and mint leaves obtained from conventional, organic, and hydroponic farming methods showed variations. The results obtained for total mesophilic aerobic count (TMAC) in the samples are presented in Table 1. Conventionally grown coriander leaves exhibited the highest TMAC, with an average of 4.55×10^9 CFU g^{-1} (colony forming unit g^{-1}). The results for TMAC in all of the conventionally grown LVs exceeded the FSSAI limits (10^5 CFU g^{-1}). Organically grown LVs had intermediate TMAC values in the range of 10^4 to 10^5, while hydroponically grown LVs consistently exhibited the lowest values of TMAC in the range of 10^3.

As evident in Table 2, conventionally grown coriander leaves showed the highest total coliform count (TCC), with an average of 4.06×10^6 CFU g^{-1}, while organic and hydroponic coriander leaves had undetectable levels (<1 CFU g^{-1}). However, the differences were statistically significant ($p < 0.05$). The TCC results for conventionally grown coriander leaves exceeded the FSSAI permissible limit (10^2 CFU g^{-1}). Additionally, conventionally grown coriander from Muhana Mandi (1.05×10^{10} CFU g) was the the only sample positive for *Escherichia coli* (*E. coli)* O157. The high microbial load is likely due to poor sanitation, sewage water use, synthetic fertilizers, and inadequate handling at the congested market (Singh and Gupta, 2019; Mohanapriya *et al.,* 2024).

TCC followed a similar trend, with conventionally grown coriander leaves exhibiting the highest levels (up to 1.20×10^7 CFU g^{-1}), while both organic and hydroponic samples showed negligible counts (<1 CFU g^{-1}). High contamination in conventional produce is linked to irrigation with untreated sewage, soil quality, and poor post-harvest handling (Mohanapriya *et al.,* 2024; Yadav *et al.,* 2025).

Table 1. Total mesophilic aerobic count for leafy vegetables grown using conventional, organic and hydroponic farming methods

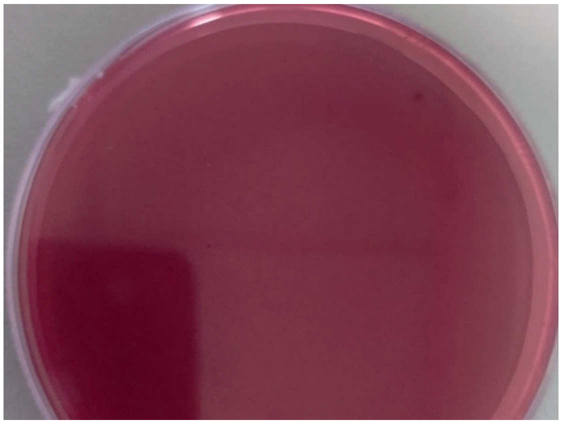
Type of the samples		Total mesophilic aerobic count (CFU g-1)			p-value
		Conventional	Organic	Hydroponic	
Coriander leaves	Market/Farm 1	1.05×10^{10}	2.05×10^{4}	1.69×10^{3}	0.000*
	Market/Farm 2	3.14×10^{9}	6.54×10^{4}	1.04×10^{3}	
	Market/Farm 3	1.92×10^{7}	1.98×10^{5}	1.31×10^{3}	
	Average	**4.55**$\times10^{9}$	$\mathbf{9.46\times10^{4}}$	$\mathbf{1.35\times10^{3}}$	
Fenugreek leaves	Market/Farm 1	1.93×10^{8}	4.32×10^{5}	1.99×10^{3}	0.000*
	Market/Farm 2	1.38×10^{7}	7.54×10^{4}	1.43×10^{3}	
	Market/Farm 3	1.64×10^{7}	4.98×10^{4}	3.64×10^{3}	
	Average	$\mathbf{7.44\times10^{7}}$	$\mathbf{1.86\times10^{5}}$	$\mathbf{2.35\times10^{3}}$	
Mint leaves	Market/Farm 1	1.59×10^{7}	1.08×10^{5}	1.14×10^{3}	0.000*
	Market/Farm 2	2.32×10^{6}	2.63×10^{5}	1.45×10^{3}	
	Market/Farm 3	1.65×10^{6}	8.92×10^{4}	1.89×10^{3}	
	Average	$\mathbf{6.62\times10^{6}}$	$\mathbf{1.53\times10^{5}}$	$\mathbf{1.49\times10^{3}}$	

*Significant at $p < 0.05$

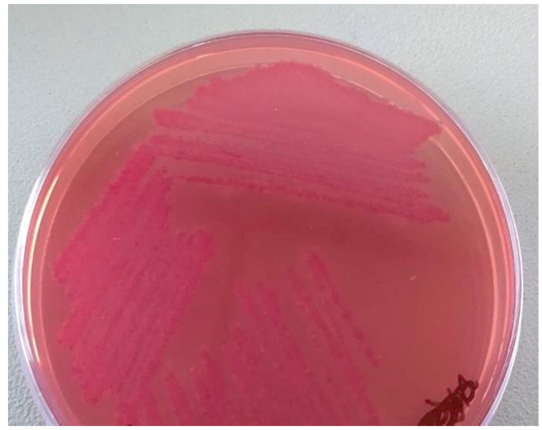
Table 2. Total coliform count and *E. coli* results for leafy vegetables grown using conventional, organic and hydroponic farming methods

Type of the samples		Total coliform count (CFU g-1)			p-value	*E. coli 0157*
		Conventional	Organic	Hydroponic		
Coriander leaves	Market/Farm 1	1.20×10^{7}	<1	<1	0.000*	Positive
	Market/Farm 2	1.34×10^{5}	<1	<1		Negative
	Market/Farm 3	1.96×10^{4}	<1	<1		Negative
	Average	$\mathbf{4.06\times10^{6}}$	**<1**	**<1**		
Fenugreek leaves	Market/Farm 1	9.63×10^{2}	<1	<1	0.000*	Negative
	Market/Farm 2	8.41×10^{2}	<1	<1		Negative
	Market/Farm 3	1.09×10^{3}	<1	<1		Negative
	Average	$\mathbf{9.64\times10^{2}}$	**<1**	**<1**		
Mint leaves	Market/Farm 1	5.96×10^{2}	<1	<1	0.000*	Negative
	Market/Farm 2	6.31×10^{2}	<1	<1		Negative
	Market/Farm 3	5.75×10^{2}	<1	<1		Negative
	Average	$\mathbf{6.01\times10^{2}}$	**<1**	**<1**		

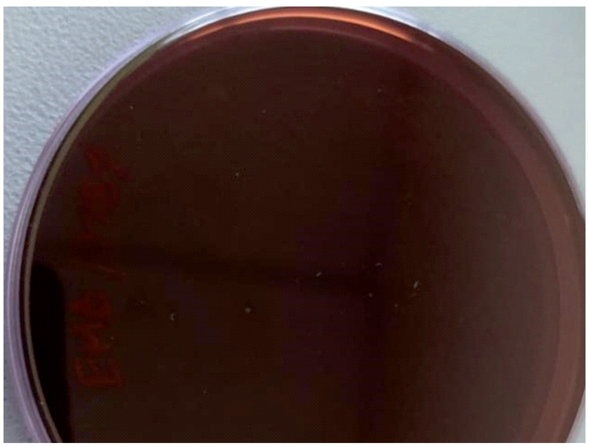
*Significant at $p < 0.05$



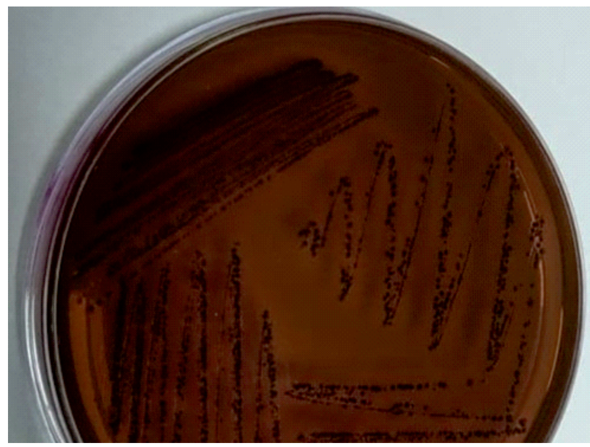
MacConkey Agar (negative)



MacConkey Agar (Positive)



Eosin Methylene Blue Agar (negative)



Eosin Methylene Blue Agar (Positive)

Plate 1. *E. coli* results for isolation test on MacConkey and Eosin methelene blue agar plates

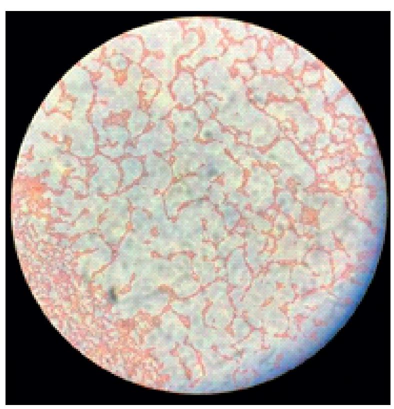


Plate 2. Microscopic image of *E. coli* strain

In addition, a clear farm-wise variation was observed across all three LVs and farming methods. Among the conventional samples, Market 1 consistently exhibited the highest microbial contamination across parameters, with TMAC of 1.05×10^{10} CFU g^{-1} in coriander, 1.93×10^{8} CFU g^{-1} in fenugreek, and 1.59×10^{7} CFU g^{-1} in mint, accompanied by elevated TCC (1.20×10^{7}, 9.63×10^{2}, and 5.96×10^{2} CFU g^{-1}, respectively). Markets 2 and 3 showed comparatively lower but still substantial microbial loads. In contrast, organic farms displayed moderate TMAC (2.05×10^{4} to 4.32×10^{5} CFU g^{-1}) and undetectable TCC (<1 CFU g^{-1}) across all leafy vegetables, while hydroponic farms consistently recorded the lowest microbial counts, with TMAC ranging from 1.04×10^{3} to 3.64×10^{3} CFU g^{-1} and TCC <1 CFU g^{-1}, showing negligible contamination in all samples.

Microbial loads in organically grown LVs were slightly lower than conventional but higher than hydroponic LVs. While organic farming uses natural fertilizers that reduced some risks, contaminated irrigation, organic amendments, and animal proximity can still contribute to contamination (Osafo *et al.,* 2022). However, hydroponically grown LVs had the lowest microbial counts, likely due to the absence of soil, controlled environment, and soil-less nutrient solutions, which minimize exposure to pathogens like *E. coli* and *Salmonella*. Good agricultural practices, including equipment sterilization and UV-C treatment, further reduced microbial growth (Perez *et al.,* 2024).

This study showed that the farming methods significantly affected food safety. However, the microbial load and the nature of the microbes majorly depend on conditions such as the quality of the manure used, the quality of the irrigation water, and the handling and transportation conditions. Good agricultural practices (GAP) and good handling practices (GHP) are essential to control contaminants, as neither organic nor hydroponic methods were found entirely free of microbial risks. Market conditions further influenced contamination, with conventionally grown LVs sourced from crowded Jaipur markets, while organic and hydroponic samples came directly from farms. As this study assessed only consumer-level contamination, it is recommended that future research should adopt a more comprehensive approach by investigating microbial risks across the entire supply chain, considering both farm-level practices and market conditions, to ensure safe and sustainable food production.

REFERENCES

Anonymous, 1976. Bureau of Indian Standards (BIS), IS 5887-1: 1976 Methods for detection of bacteria responsible for food poisoning: Part 1 isolation, identification, and enumeration of *Escherichia coli*. 1976. https://law.resource.org/pub/in/bis/S06/is.5887.1.1976.pdf.

Anonymous, 2012a. Bureau of Indian Standards (BIS), IS 5402: 2012 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of micro-organisms - Colony count technique at 30°C. https://law.resource.org/pub/in/bis/S06/is.5402.2012.pdf

Anonymous, 2012b. Bureau of Indian Standards (BIS), IS 5401 (Part 1): 2012 Microbiology General guidance for the enumeration of coliforms (First revision). https://law.resource.org/pub/in/bis/S06/is.5401.1.2012.pdf.

Anonymous, 2023a. Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO), Prevention and control of microbiological hazards in fresh fruits and vegetables. FAO, Rome. https://openknowledge.fao.org/server/api/core/bitstreams/151c9dda-508b-4b70-972a-85725eb12145/content

Anonymous, 2023b. Food Safety and Standards Authority of India (FSSAI), Appendix B: Microbiological requirements (Version 11 September 2023). FSSAI, New Delhi. https://www.fssai.gov.in/upload/uploadfiles/files/Appendix%20B.pdf

Bharai, R.A.H., 2024. Role of green food in nutritional security. Int. J. Sci. Res. **13**(1):384-386.

Crowther, T., R. Rappuoli, C. Corinaldesi, R. Danovaro, T.J. Donohue, J. Huisman, L.Y. Stein, J.K. Timmis, K. Timmis, M.Z. Anderson , 2024. Scientists call to action: Microbes, planetary health, and the Sustainable Development Goals. Cell **187**(19):5195-5216.

HiMedia, 2024. Microbiological testing standards for food safety. Accessed December 13, 2024. https://www.himedialabs.com/in/coasdstds.

Kalpana, P., S. Yasobant, D. Saxena and C. Schreiber, 2024. Microbial contamination and antibiotic resistance in fresh produce and agro-ecosystems in South AsiaA systematic review. Microorganisms. **12**(11):2267.

Kashyap, P. and M. Jain, 2024. Concentration and non-carcinogenic risk assessment of heavy metals in leafy vegetables among different farming practices. Int. J. Environ. Anal. Chem.: 123. doi:10.1080/03067319.2024.2420828.

Kashyap, P. and M. Jain, 2025. Organic farming: emerging practices, effect on environment and nutrition. Nature Environ. Pollut. Technol. **24**(S1):137-148.

Mohanapriya, R., V. Paranidharan, S. Karthikeyan and D. Balachandar, 2024. Surveillance and source tracking of foodborne pathogens in the vegetable production systems of India. Food Control. **162**:110427.

Mritunjay, S.K. and V. Kumar, 2017. A study on prevalence of microbial contamination on the surface of raw salad vegetables. 3 Biotech. **7**(1):13.

Osafo, R., G.I. Balali, P.K. Amissah-Reynolds, F. Gyapong, R. Addy, A. Nyarko and P. Wiafe, 2022. Microbial and parasitic contamination of vegetables in developing countries and their food safety guidelines. J. Food Qual. **1**:4141914.

Pawar, N.P., V.G. Atkare, U. Dongarwar and S. Rananaware, 2024. Effect of feeding of hydroponic maize fodder on milk production and economics of feeding of crossbreed cows. J. Soils and Crops, **35**(1):143-145.

Perez, S.L., R.B. Ferro, B. Corrêa, R. Casarin, T.Q. Corrêa, K.C. Blanco and V.S. Bagnato, 2024. Enhanced vegetable production in hydroponic systems using decontamination of closed circulating fluid. Sci. Rep. **14**(1):602.

Sai Lakshmi, K.S., J. Lakshmi, K. Lakshmi and G. Manasa, 2020. Consumption pattern of green leafy vegetables among different income groups in Guntur town of Andhra Pradesh. Andhra Agric. J. **67**(3):219-222.

Sarkar, T., M. Salauddin, S. Roy, R. Chakraborty, M. Rebezov, M.A. Shariati, M. Thiruvengadam and K.R.R. Rengasamy, 2023. Underutilized green leafy vegetables: frontier in fortified food development and nutrition. Crit. Rev. Food Sci. Nutr. **63**(33):11679-11733.

Singh, S. and P. Gupta, 2019. Possible effects of sewage farming on ground water quality in Muhana Mandi, Jaipur. Methods. **5**(1):56-61.

Yadav, K.K., R. Manaware, R. Chauhan, P. Tripathi and P. Yadav, 2025. Emerging trends and challenges of soil contamination and its remedies. J. Soils and Crops, **35**(2):18.

Rec. on 20.09.2025 & Acc. on 10.10.2025