

Analysis of Microbial Contaminants found in Mints and Lemongrass sold by Road Traffic Vendors in Kano Metropolis

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ABSTRACT

Food is a basic necessity for human survival, but it is still the vehicle of transmission of food borne diseases. This paper investigated microbial contaminants of mints and lemongrass sold by road traffic vendors in Kano metropolis, Nigeria. Samples of mints and lemongrass were purchased from vendors at major traffics in Kano. These samples were taken to Microbiology Laboratory, Bayero University Kano to analyze the microbial contaminants present in the samples. The total bacterial and fungal count in 30% of the samples were significantly high, exceeding World Health Organization (WHO) limit of 100 CFU/g. Bacteria such as *Staphylococcus aureus* and *Salmonella typhi*, were commonly isolated. *Staphylococcus aureus* was isolated in all of the samples while *Salmonella typhi*, was isolated in 20% of the samples. Fungal contamination was also observed with *Rhizopus* spp being the predominant genus. *Fusarium* spp and *Aspergillus* spp were also isolated from the samples. The study helped identified the possible sources of contamination, as such users should ensure to wash the leaves properly before usage, and further research should be carried out to ascertain whether the contamination is bioaccumulated within the plant.

Keywords: Microbial Contamination, Mints and Lemongrass, Food Safety, Kano Metropolis, Public Health Risks

INTRODUCTION

In recent times, there has been increased consumption of mints and lemongrass due to increasing awareness of their numerous benefits. Herbal plants such as mint leaves, lemon grass and so on represent an important class of various traditional medicine system. They are increasingly used in the primary health care intervention both in developed and developing countries (Achibi, 2020). According to the World Health Organization, nearly 70-80% of the world population still primarily relies on non-conventional medications mostly derived from herbal plants (WHO, 2005). This has led to the increased cultivation and sale of these herbs. They are now being sold in almost every road traffic within Kano Metropolis.

The increasing population in Kano State has led to a rapid urbanization and industrialization (Uzonu, 2021). In order to improve crop productivity through improved irrigation, local farmers resorted to utilizing immense amount of domestic and industrial wastewater for watering mints, lemongrass and other crops. They also use undecomposed manure and pesticides for their production, which negatively affects the health of the population and the environment. Unhygienic harvesting

methods, as well as hands contaminated with faeces and sprinkling of water on mints and lemongrass to make them fresh by the traffic vendors contributes to contamination.

In Nigeria, over 200,000 people die from food-borne illness annually caused by contaminated food and the economic burden associated with food-borne illnesses is around US\$ 3.6 billion per annum (Onyeaka, 2021) accounting for 17-25% of the estimated cost of all illness (Onyeaka *et al.*, 2016). Although Nigeria has no official food-borne surveillance system, the local government health system profile in Nigeria revealed food-borne illnesses as the leading cause of death accounting for 25% mortality (Igbiosa *et al.*, 2021).

This research provides a comprehensive understanding on how the microbial contaminants found in mints and lemongrass sold by road traffic vendors in Kano Metropolis can contribute to the high rate of mortality due to food-borne diseases in the country. This understanding can then be useful in developing targeted strategies for reducing these contaminants. Data and information provided by this research can be used in developing agricultural, environmental and health policies for Kano State and improve existing ones. It will also enable policy makers regulate the production, marketing and handling of these leaves for public health protection.

MATERIALS AND METHODS

Study Area

Kano metropolis is located between latitudes 11°50' and 12°07' North of the Equator and longitudes 8°22' and 8°47' East of Great Meridian. It comprises of eight local government, four of which formed the significant portion in terms of land mass in the vestigial ancient city wall enclosure, these core local government areas are Dala, Fagge, Gwale and Kano Municipal. Nassarawa and Tarauni local governments formed the nucleus of Metropolitan and enveloped by Kumbotso and Ungogo. There are hardly any pronounced physical boundaries between the local government areas. Kano metropolis is located at the centre of the West African larges plain, Hausa plain and the capital of *Kasar Kano* (Mohammed, 2015).

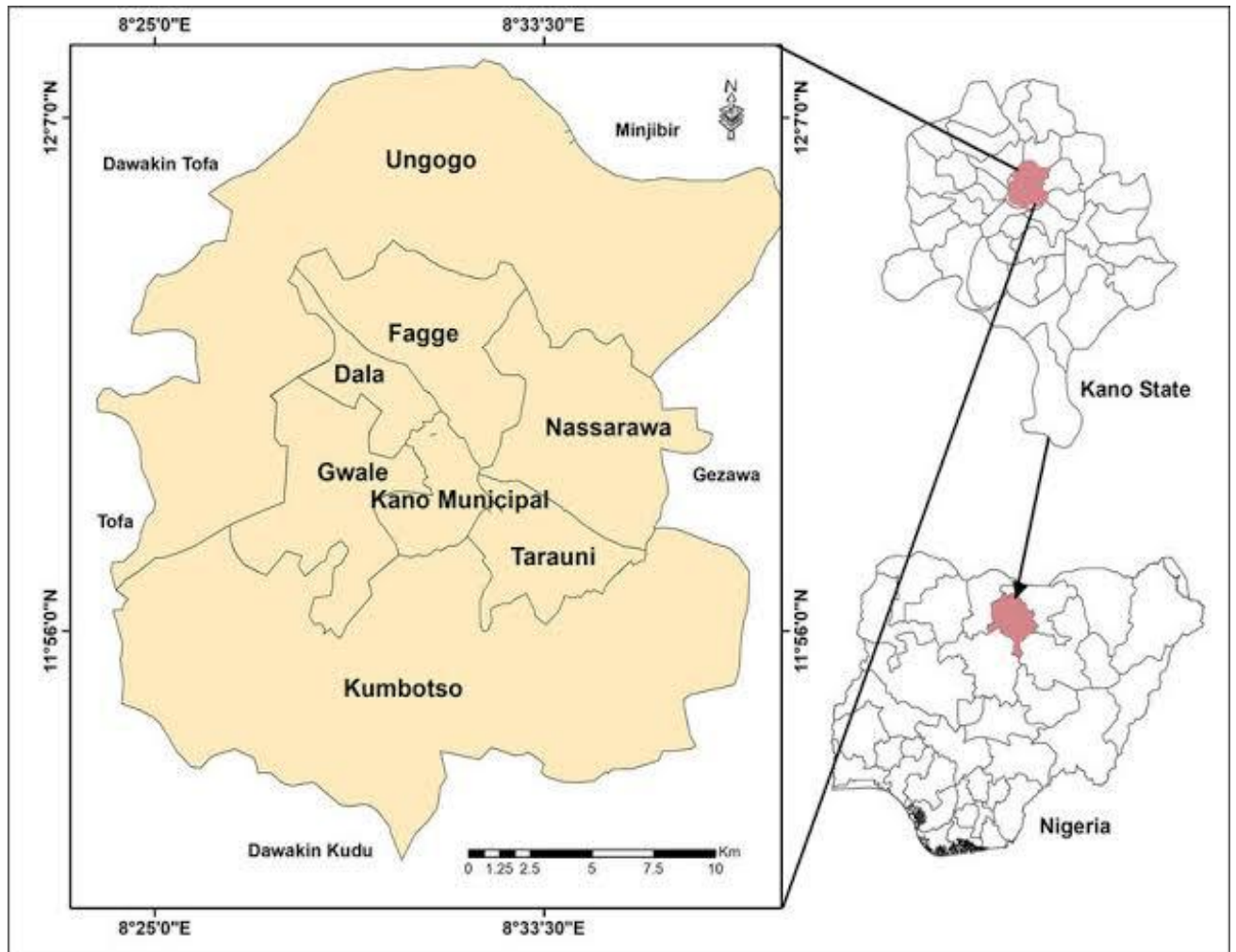


Figure 1: Kano Metropolis, Kano State Nigeria.
Source: Adopted from Umar and Naibbi, 2020.

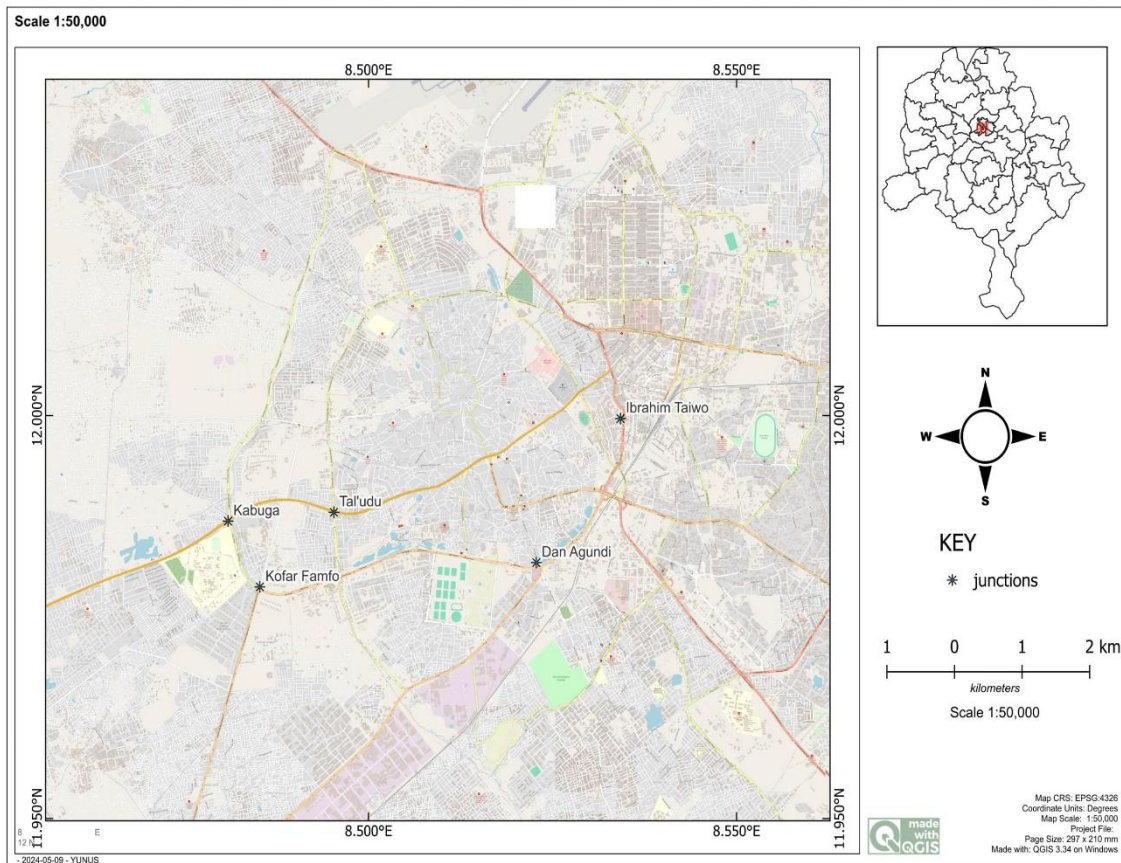


Figure 2: Sampling Locations.

Source: Geographic Information Systems (GIS) Lab, Bayero University Kano

Method of Sample Collection

Since the research aims to evaluate the microbial contaminants of mints and lemongrass sold by road traffic vendors within Kano Metropolis, an experimental research approach was chosen in two stages. This research adapts purposive sampling and simple random sampling techniques. The scope of this research was selected through purposive sampling. Road traffic vendors were specifically targeted because they are the main sellers of these leaves. The leaves were collected through random sampling by choosing the vendors at random. This technique was adopted to give each vendor an equal chance of being involved in the study and to have unbiased results. The study used ten (10) samples of mints and lemongrass collected from road traffic vendors along Tal-udu, Kabuga, Kofar Famfo, Danagundi and Kwari junctions. The samples collected were transported in a clean polythene bag to the Microbiology Laboratory in the Department of Microbiology, Bayero University, Kano.

Sample Preparation and Treatment

The samples were washed thoroughly with sterile distilled water and stored temporarily in the freezer and later blended using electrical blender to obtain fine particles of the samples. 25g of the food sample was weighed and homogenized in sterile phosphate-buffered saline (PBS) solution. The homogenate was mixed by shaking and 10mL of this mixture was transferred into a test tube and was labeled appropriately in line with the Food and Agriculture Organization (FAO) procedure of 1979.

Digestion of Samples and Laboratory Analysis

Serial Dilution: Serial dilutions of the sample were prepared using sterile dilution tubes. From the homogenate prepared above, 1ml of the mixture was transferred with a pipette into the first dilution tube containing 9ml of sterile peptone water and it was mixed vigorously; it was then serially diluted to four other test tubes. This procedure was carried out for all the samples according to FAO (1979) procedure.

Plate Count for Bacteria Enumeration: This was carried out by pour plating technique after serial dilution (Egboh and Emeshih, 2007). Using a fresh syringe, 1ml from each dilution was transferred into appropriately labeled duplicate petridishes. This was followed by pouring cooled molten nutrient agar in each petridish, homogenized by swirling and allowed to solidify. Finally, the plates were incubated at 37°C for 24 hours. Following incubation, plates that contain between 30-300 colonies were selected and the average of the colonies from the two plates were multiplied by the inverse of the dilution factor inline with FAO (1979) procedure.

Plate Count for Fungal Enumeration: The same procedure as in above was used but potatoes dextrose agar (PDA) was used for the culture. The temperature for incubation was normal room temperature for 7 days depending on the good growth of the fungi. During the incubation of the plated samples, developing fungal colonies were counted and recorded, then sub-cultured continuously on fresh PDA plates to obtain pure cultures of the isolates. Fungal isolates were identified based on growth pattern, colour of mycelia and microscopic examinations of the fungi (IMI, 1992; Barnett and Hunter, 1998).

Gram Staining: Bacteria isolates were subjected to gram staining using Nutrient Agar (N.A.), Mannitol Salt Agar (M.S.A.) and Salmonella and Shigella Agar (S.S.A.) for further identification.

Biochemical Tests: Biochemical tests such as catalase, oxidase, and sugar fermentation tests were performed for bacterial identification. They were observed for colour, shape, size, and texture.

I. Catalase Test

Catalase test was carried out by applying one drop of 3% hydrogen peroxide on a microscopic slide. Bacteria was transferred with a plastic loop to the H₂O₂ solution. A catalase positive bacteria produces oxygen gas in form of bubbles which shows the bacterium has a catalase.

II. Oxidase Test

A small piece of filter paper was soaked in 1% Kovács oxidase reagent and dried. A sterile wire loop was used to pick a well-isolated colony from the fresh (24 hour culture) bacterial plate and rubbed onto the treated filter paper (Cheesebrough, 2006).

III. Urease Test

The surface of the urea agar slant was streaked with a portion of a well- isolated colony. The cap was left on loosely and the tube was incubated at 37°C in ambient air for 48 hours. Development of a pink colour was examined for as long as 7 days. (Cheesebrough, 2006).

Fungal Microscopic Examination: Fungal isolates were examined under a microscope for hyphal morphology and spore characteristics to identify the fungal species present in the samples.

Method of Analyzing Data

The data obtained from the lab were presented in tables, the outcome was compared to the World Health Organization (WHO) and Food and Agricultural Organization (FAO) standards.

RESULTS AND DISCUSSIONS

Enumeration of Bacteria and Fungi from the Samples

Table 1 below shows the total bacteria and fungal counts for the collected samples in Colony Forming Unit per gram (CFU/g). The results indicate that some of the samples analyzed contain bacterial and fungal contaminants. The results of the plate count for the lemongrass samples indicate that the lemongrass sample from Kofar Famfo traffic has the highest bacteria and fungal count of 170×10^5 CFU/g while the lemongrass sample from Ibrahim Taiwo traffic has the lowest bacteria and fungal count of 89×10^5 CFU/g. The lemongrass samples from Tal-udu and Kabuga traffic have bacteria and fungal count less than 30 thus they are too few to count and practically negligible. This is different from the findings of Habeeb, *et.al.*, (2022) where the bacterial load present in the samples were all below 10×10^5 CFU/g for ready to eat vegetables sold in Yankaba market, Kano. The total bacterial and fungal count in 30% of the samples (Lemongrass sold at Kofar Famfo, road traffic, Lemongrass sold at Dan Agundi road traffic and Mint sold at Ibrahim Taiwo road traffic) were significantly high, exceeding WHO/FAO limit of 100 CFU/g. This can be attributed to different factors including pre-harvest and post-harvest processes such as use of wastewater for irrigation, harvesting and storage equipment, transportation containers and vehicles, rinse water used by the vendors, sprinkling of contaminated water on the leaves to keep them fresh or using contaminated hands while selling, etc. High bacterial and fungal loads in these leaves can lead to varying health implications depending on the bacteria or fungi present in the the samples.

Table 1: Bacteria and Fungi Enumeration Result.

Samples	Road Traffic	10^{-1} (CFU/g)	10^{-2} (CFU/g)	10^{-3} (CFU/g)	10^{-4} (CFU/g)	10^{-5} (CFU/g)
Lemongrass	Tal-udu	TNC	241×10^2	110×10^3	TFC	TFC
	Kabuga	TNC	210×10^2	175×10^3	82×10^4	TFC
	Kofar Famfo	TNC	TNC	250×10^3	200×10^4	170×10^5
	Dan Agundi	TNC	211×10^2	193×10^3	150×10^4	111×10^5
	Ibrahim Taiwo	TNC	242×10^2	195×10^3	132×10^4	89×10^5
Mint	Tal-udu	TNC	194×10^2	121×10^3	82×10^4	47×10^5
	Kabuga	TNC	121×10^2	50×10^3	TFC	TFC

Kofar Famfo	TNC	189x10 ²	140x10 ³	92x10 ⁴	TFC
Dan Agundi	TNC	80x10 ²	61x10 ³	40x10 ⁴	TFC
Ibrahim Taiwo	TNC	TNC	287x10 ³	192x10 ⁴	110x10 ⁵

Key: TNC = Too Numerous To Count, TFC = Too Few To Count

Observing Samples Through Different Culture Media

Table 2 shows the result of the samples observed through different culture media. Nutrient Agar, a general purpose bacteria nutrient medium was used to confirm the presence of bacteria in all the samples (*Staphylococcus aureus* and *Salmonella typhi*). Potato Dextrose Agar was used do confirm the presence of fungi in all the samples. (*Rhizopus* spp, *Fusarium* spp and *Aspergillus* spp). Eosin Methylene Blue was used as a selective media to confirm the presence or absence of *E. coli* in the samples. There was no growth in the media indicating the absence of fecal contamination in the samples.

Salmonella and Shigella Agar, a differential selective media was used to confirm the presence of *Salmonella typhi* in the samples. Mannitol Salt Agar was used to confirm and isolate the presence of *Staphylococcus aureus* in the samples.

Table 2: Culture Media Observations.

Samples	Road Traffic Location	N.A.	P.D.A.	E.M.B.	S.S.A.	M.S.A.	L.B.
Lemongrass	Tal-udu	Growth	Growth	No Growth	No Growth	Growth	No Gas Formed
	Kabuga	Growth	Growth	No Growth	No Growth	Growth	No Gas Formed
	Kofar Famfo	Growth	Growth	No Growth	No Growth	Growth	No Gas Formed
	Dan Agundi	Growth	Growth	No Growth	No Growth	Growth	No Gas Formed
	Ibrahim Taiwo	Growth	Growth	No Growth	No Growth	Growth	No Gas Formed
Mint	Tal-udu	Growth	Growth	No Growth	No Growth	Growth	No Gas Formed
	Kabuga	Growth	Growth	No Growth	No Growth	Growth	No Gas Formed

Kofar Famfo	Growth	Growth	No Growth	No Growth	Growth	No Gas Formed
Dan Agundi	Growth	Growth	No Growth	No Growth	Growth	No Gas Formed
Ibrahim Taiwo	Growth	Growth	No Growth	No Growth	Growth	No Gas Formed

Key: N.A. = Nutrient Agar, P.D.A. = Potato Dextrose Agar , E.M.B = Eosin Methylene Blue, S.S.A. = Salmonella and Shigella Agar , M.S.A. = Mannitol Salt Agar, L.B. = Lactose Broth.

Gram Reaction of Bacterial Isolates

The isolated bacteria were gram stained to differentiate it into gram positive and gram negative bacteria. *Staphylococcus aureus* was observed to have a purple colouration after the gram staining process which indicates a positive cocci result, this is because it has cell walls that contains thick layers of peptidoglycan which retains the crystal violet it was stained with while the *Salmonella typhi* was observed to have a red colour after the gram staining process indicating a negative result, this is because it has cell walls containing thin layers of peptidoglycan and high lipid content enabling it to retain the colour of the counter stain. It is shown in the table 3 below.

Table 3: Gram Staining Result.

Samples	Road Traffic Location	N.A.	M.S.A.	N.A. and M.S.A. Identification	S.S.A.	S.S.A. Identification
Lemongrass	Tal-udu	Gram Positive Cocci	Gram Positive Cocci	<i>Staphylococcus aureus</i>	N/A	N/A
	Kabuga	Gram Positive Cocci	Gram Positive Cocci	<i>Staphylococcus aureus</i>	N/A	N/A
	Kofar Famfo	Gram Positive Cocci	Gram Positive Cocci	<i>Staphylococcus aureus</i>	Gram Negative Rod	<i>Salmonella typhi</i>
	Dan Agundi	Gram Positive Cocci	Gram Positive Cocci	<i>Staphylococcus aureus</i>	N/A	N/A
	Ibrahim Taiwo	Gram Positive Cocci	Gram Positive Cocci	<i>Staphylococcus aureus</i>	N/A	N/A

Mint	Tal-udu	Gram Positive Cocci	Gram Positive Cocci	<i>Staphylococcus aureus</i>	N/A	N/A
	Kabuga	Gram Positive Cocci	Gram Positive Cocci	<i>Staphylococcus aureus</i>	N/A	N/A
	Kofar Famfo	Gram Positive Cocci	Gram Positive Cocci	<i>Staphylococcus aureus</i>	N/A	N/A
	Dan Agundi	Gram Positive Cocci	Gram Positive Cocci	<i>Staphylococcus aureus</i>	N/A	N/A
	Ibrahim Taiwo	Gram Positive Cocci	Gram Positive Cocci	<i>Staphylococcus aureus</i>	Gram Negative Rod	<i>Salmonella typhi</i>

Key: N.A. = Nutrient Agar, M.S.A. = Mannitol Salt Agar, S.S.A. = Salmonella and Shigella Agar, N/A= Not Applicable

Biochemical Characterization of Bacterial Isolates

Table 4 shows the biochemical characteristics of gram positive bacteria isolated from the test samples. *Staphylococcus aureus* was the only gram positive bacteria present. It was catalase positive, coagulase positive and oxidase negative. *Salmonella typhi* was the only gram negative bacteria present. It was catalase positive, coagulase positive and oxidase negative. This is similar to the findings of Rane, 2011; Ajayi and Oluwoye, 2015; Balali *et al.*, 2020; Erhirhie *et al.*, 2020 and Habeeb *et al.*, 2022 where *Staphylococcus aureus* and *Salmonella typhi* were isolated in all the studies. Presence of pathogens in food products imposes potential hazard for consumers and causes grave economic loss and loss in human productivity via food-borne disease. Symptoms of *Staphylococcus* food disease include nausea, vomiting, and abdominal cramps with or without diarrhoea (Kadariya *et al.*, 2014). In *Salmonella* food disease, most people develop diarrhoea, fever and stomach (abdominal) cramps within 8 to 72 hours after exposure. In some cases, diarrhoea can cause severe dehydration and requires prompt medical attention. Life-threatening complications may develop if the infection spreads beyond the intestines.

Table 4: Biochemical Test Result for Isolated Bacteria

Suspected Organism	Catalase Test	Coagulase Test	Oxidase Test	Inference
<i>Staphylococcus aureus</i>	+	+	-	<i>Staphylococcus aureus</i>
<i>Salmonella typhi</i>	+	+	-	<i>Salmonella typhi</i>

Key: + = Positive, - = Negative.

Cultural Characteristics and Microscopic Features of Isolates

The microscopic and cultural features of the isolated fungi *Rhizopus* spp, *Fusarium* spp and *Aspergillus* spp were represented in Table 5. The *Rhizopus* spp was characterized by a body of branching mycelia composed of three types of hyphae (stolon, rhizoid and unbranching sporangiophores). The *Fusarium* spp was observed to be fast growing producing both macro and micro conidia from slender phialides and is sickle shaped. *Aspergillus* spp was characterized by the formation of cylindrical shaped phialides onto the surface of the vesicle at the apex of unbranched conidiophore. This is similar to the findings of Oluwafemi (2013) and Erhirhie *et al.*, 2020, where *Rhizopus* spp,, *Fusarium* spp and *Aspergillus* spp were isolated in their studies alongside other fungal contaminants. This can also be attributed to several pre-harvest and post-harvest processes, like the bacterial contaminants found in these samples as discussed above.

Aspergillus niger has been associated with otomycosis (Bojanavic *et al.*, 2022), cutaneous infections (Merad *et al.*, 2021) and pulmonary disease (Ashby *et al.*, 2023). *Fusarium* species cause a broad spectrum of infections in humans, including superficial infections such as keratitis and onychomycosis, as well as locally invasive and disseminated infections (Lainhart, 2018). Invasive and disseminated infections occur almost exclusively in severely immunocompromised patients, particularly among those with prolonged and profound neutropenia and/or severe T cell immunodeficiency. Among patients with hematologic malignancy, the infection predominates during periods of neutropenia, typically among patients with leukemia receiving induction chemotherapy. *Fusarium* species may also cause allergic diseases, such as sinusitis in immunocompetent individuals (Singh, 2019) and mycotoxicosis following ingestion of food contaminated by toxin-producing *Fusarium* species (Ekwomadu & Mwanza, 2023). *Rhizopus* species may cause respiratory infections, sinusitis and otomycosis.

Table 5: Fungal Plates Microscopy

Samples	Road Traffic Location	Microscopic Observation	Identification By Chart
Lemongrass	Tal-udu	Stolons and Pigmented Rhizoids	<i>Rhizopus</i> spp
	Kabuga	Stolons and Pigmented Rhizoids	<i>Rhizopus</i> spp
	Kofar Famfo	Sickle-Shaped With Two Fusiforms	<i>Fusarium</i> spp
	Dan Agundi	Elongated Apical Cell Stolons and Pigmented Rhizoids	<i>Rhizopus</i> spp
	Ibrahim Taiwo	Unbranched Conidiophore Which is Non-Septate, Rough and Hyaline	<i>Aspergillus</i> spp

Mint	Tal-udu	Stolons and Pigmented Rhizoids	<i>Rhizopus</i> spp
	Kabuga	Stolons and Pigmented Rhizoids	<i>Rhizopus</i> spp
	Kofar Famfo	Stolons and Pigmented Rhizoids	<i>Rhizopus</i> spp
	Dan Agundi	Sickle-Shaped With Two Fusiforms	<i>Fusarium</i> spp
	Ibrahim Taiwo	Elongated Apical Cell Unbranched Conidiophore Which is Non-Septate, Rough and Hyaline	<i>Aspergillus</i> spp

CONCLUSION

This research revealed the presence of various contaminants at varying levels in mints and lemongrass sold by road traffic vendors in Kano Metropolis, Nigeria. These contaminants present significant risks to humans. The current practices observed for handling these leaves fall short of safeguarding human health as most of the contaminants present are above the recommended safe limits of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) for human consumption.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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