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Microbiological Assessment of Some Vegetables Obtained from Irrigated farms within Kaduna Metropolis

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Abstract: *The microbiological assessment of fresh vegetables obtained from irrigated farms has revealed that it is the potential route for transmitting pathogens to humans. This study is aimed at investigating microorganisms commonly found in fresh vegetable farms in Kaduna north western Nigeria. Enumeration and characterization of Total bacterial count, Coliform count and Fungal isolates were determine using a standard methods. The isolated pathogenic bacteria genera and fungi from irrigated fresh vegetable samples include Staphylococcus aureus,with 31.7% occurrenceEschericiacoli,had 28.6% occurrence, Pseudomonas aeruginosa,15.9 %occurrence while Bacillus spp,had 23.8%. For the fungal isolates,Aspergillusspp had 33.8%Penicilliumspp, 33% occurrenceFusariumspp had 25.6% while SacharomycessppandRhyzopusspp had 3.6% occurrencesrespectively. The bacterial counts coliform counts and the fungal counts in the fresh vegetables were observed to be higher than acceptable limits. The count of the coliform in the irrigated a vegetable suggests faecal contamination and this raise the possibility of the presence of pathogenic microorganisms in the fresh vegetables and a threat to public health.*

Key Words:*Microbiological assessment, Coliforms, Irrigation, Vegetables*

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I. Introduction

Irrigation is the artificial application of water to soil food crop farming. It is used to assist in the growing of agricultural produce since nutrients are made available to plants in liquid form for their maximal growth and development[1]

Vegetables are an extraordinary dietary source of nutrient, micronutrients, vitamins and fiber for humans and well-balanced diets, rich in fruits and vegetables, are especially valuable for their ability to prevent vitamins C and vitamin A deficiencies and are also reported to reduce the risk of several diseases[2]. Vegetables are widely exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing. They therefore harbor range of microorganisms including plants and animal pathogen[3]. Differences in microbial profiles of various vegetables result largely from unrelated factors such as resident micro flora in the soil, application of non-resident micro-flora via animal manures, sewage or irrigated water, transportation and handling by individual retailers[4]. Vegetables can be contaminated while growing in the fields as a result of organic fertilizer.

The lack of effective antimicrobial treatments at any step from planting to consumption means that pathogens introduced at any point may be washed or treated specifically to minimize microbial load. Vegetables are mostly annual crops belonging to the group of plant called horticultural crops which are diverse in nature[5]. Microbiological, irrigated vegetables are found to be highly contaminated with bacterial that are harmful to both plants and animals including man[6]. Consumption of these types of vegetables unhygienic ally paves way for ingestion of considerable number of human pathogenic bacteria. This eventually results in establishment and manifestation of disease on the final host. The presence of cut and damage can provide an opportunity for contamination and growth of microorganism into plant tissue[7]. It is widely accepted that vegetables are important component of a healthy diet and their consumption could help prevent a wide range of disease[2]. The intake of goods should be expressed in numerical terms so that the potential public health benefits can be evaluated. It is well know that population have diverse food availability and preferences, differing dietary pattern and cultural consideration of food. Local circumstances must be carefully considered in adaptation of dietary guidelines to individual country diets and pollution[8]. This project is aimed at assessing the microbial contamination of some vegetables obtained from irrigated farms within Kaduna metropolis.

II. Materials And Methods

2.1 Sample collection and handling

A total 50 samples of vegetables were collected from different irrigated farms situated in Badiko, Kudendan, Malali,AnguwanRimi and River Kaduna. The vegetables samples includes: Cucumber (Cucumbissativus), Cabbage (Brassica olerancea), Garden egg (Solanummelonema), Carrot (Daucuscarota) and Lettuce (Lactuca sativa) each sample was collected in a sterile polythene bag and transported to the laboratory for further analysis.

2.2 Media Preparation

The media use includes Nutrient agar, for total bacterial count (TBC), EMB agar, Mac-Conkey agar for total coliform count (TCC) and Sabouraud Dextrose Agar for total fungal count (TFC) were prepared according to manufacturer's instructions.

2.2.1 Preparation of Food Homogenate and Dilution

Ten grams (10g) of each vegetables samples was weighed and soaked for 15 minutes and washed by shaking thoroughly with 225 ml of 0.1% sterile peptone water and wash homogenated with 90ml of Ringer's solution separately. They were mixed by shaking properly; 1ml of each homogenate was serially diluted into sterile tubes containing 9ml of ringer's solution and serially diluted up to 10^5 dilution factor.

2.2.2 Inoculation

Zero point one milliliter (0.1 ml) of the 3^{rd} and 4^{th} dilution was inoculated on nutrient and Eosine Methylene Blue (EMB) agar plates using spread plate technique. Inoculated plates were incubated at 37^oc and 44.5^oc respectively for 24 h. characteristics colonies which appeared were counted as total aerobic heterotrophic count for nutrient agar plate and green black with metallic sheen on EMB agar were counted as faecal coliforms. Typical colonies on EMB were inoculated into lactose broth in test tubes containing inverted Durham.s tubes and were incubated at 44^oc for 24 h for confirmatory tests. Gas and acid production confirmed faecal coliform tests [9] while the colonies on nutrient agar plates were sub cultured repeatedly on fresh sterile petri dish for pure isolates which were later transferred to slant bottles for biochemical test.

2.3 Characterization and Identification of Bacterial Isolates

2.3.1 Identification of Isolates

The isolates from nutrient, EMB agar and Macconkay agar were subcultured onto another plate aseptically using the same medium and pure isolate were obtained. These isolates were further subcultured into universal slant bottles containing nutrient agar for biochemical test. The following biochemical test was carried out for the identification of bacterial isolates. Catalase coagulase, urease, citrate, motility, indole, oxidase,Methyl red (MR), VogesProskauer (VG) sugar fermentation such as glucose, maltose, sucrose mannitol, lactose, fructose starch hydrolysis

2.4 Characterization and Identification Of Fungal Isolates

Identification of fungal isolates was based on Macroscopic, microscopicand morphology based on their color and shapes and microscopic structure such as hypea, septate, conidiophores, conidia and phialide. The technique of [10] was adopted for the identification of the unknown isolated fungi using cotton blue in lacto phenol stain. The identification was achieved by placing a drop of the stain on a clean slide with the aid of a mounting needle where a small portion of the mycelium from the fungal culture was removed and placed in a drop and lacto-phenol. The mycelium was spread very well on the slide with the aid of a needle, a cover slip was gently placed with little pressure to eliminate air bubbles the slides was then mounted and observed with the aid of x10 and x40 objectives lens respectively.

III. Results and Discussion

Table 1: Total Mean Bacterial Count of the Isolates from Different Farms within Kaduna Metropolis

s/no	Farms	Cabbage	Carrot	Lettuce	Cucumber	Garden egg
1	Kudende	6.3×10^4	7.7×10^4	8.9×10^4	8.4×10^4	5.4×10^4
2	Badiko	7.3×10^4	7.7×10^4	8.0×10^4	6.9×10^4	6.1×10^4
3	Malali	5.0×10^4	7.2×10^4	7.9×10^4	5.8×10^4	5.9×10^4
4	A/Rimi	6.2×10^4	7.2×10^4	7.5×10^4	5.9×10^4	6.1×10^4
5	River Kaduna	6.5×10^4	5.4×10^4	9.0×10^4	7.4×10^4	7.9×10^4
Total mean count		6.26×10^4	6.98×10^4	8.26×10^4	6.88×10^4	6.28×10^4

Table 2: Percentage occurrence of bacterial isolates on fresh vegetables

Organisms isolated	Occurances	Percentage (%) Occurances
Staphylococcus aureus	20	31.7
Eschericia coli	18	28.6
Pseudonasaeruginosa	10	15.9
Bacillus cereus	15	23.8

Table 3: Colonial and Morphological Characteristics of Fungi Isolated from Vegetables

S/no	Organisms isolated	Macroscopic Characteristics	Microscopic chacteristics
1	Aspergillusspp	Deep brown powdery colony	The hyphae is non-septate, conidia is globose
2	Fusariumspp	White cottony at first burned pinkish with age	Macroconidia and Microconidia from slender phialides
3	Penicilliumspp	White colonies appeared at first but turn green as cultures becomes old	Highly branched conidiospore, phialides are septate and subglobose
4	Saccharomyces spp	Milky circular colonies	Non septate hyphae with sporangia
5	Rhizopusspp	White to yellow cottony colonies	Non-septate hyphae with branched sporangiophore

Table 4: Percentage occurrence of fungal isolates on fresh vegetables

Organisms isolated	Occurances	Percentage (%) Occurances
Aspergillusspp	47	33.8
Penicilliumspp	46	33
Fusariumspp	36	25.9
Rhizopusspp	5	3.6
Sacharomycesspp	5	3.6

Table 5: Occurance of Fungi Isolated from Different Vegetables Obtained from the five Farms within Kaduna Metropolis

Farm	Samples	Aspergillusspp	Penicilliumspp	Fusariumspp	Saccharomyces spp	Rhizopusspp
Kudendan	Cabbage	2	1	-	-	-
	Cucumber	2	3	-	1	-
	Garden egg	1	-	-	-	1
	Lettuce	-	-	2	-	1
	Carrot	3	-	-	2	1
Badiko	Cabbage	2	1	2	-	-
	Cucumber	2	1	2	-	-
	Garden egg	-	1	2	1	-
	Lettuce	1	2	1	-	-
	Carrot	3	1	2	-	1
Malali	Cabbage	2	2	1	-	-
	Cucumber	-	1	-	-	1
	Garden egg	1	2	1	-	-
	Lettuce	1	2	1	-	-
	Carrot	1	-	2	-	-
A/Rimi	Cabbage	5	2	3	-	-
	Cucumber	2	3	1	-	-
	Garden egg	2	4	1	-	-
	Lettuce	5	2	4	-	-
	Carrot	3	2	1	-	-
R/ Kaduna	Cabbage	2	3	4	-	-
	Cucumber	1	4	1	-	-
	Garden egg	2	3	1	-	-
	Lettuce	1	4	1	-	-
	Carrot	3	2	3	-	-

Key:spp (species), A/Rimi (AnguwanRimi), R/Kaduna (River Kaduna).

IV. Discussions

In this study, it was observed that almost all the ready to eat vegetables examined had bacterial counts above the acceptable limit and it is microbiologically unacceptable. The international microbiological standard recommended limits of bacterial contamination for food range less than 10^3cfu/g for total plate count. The high bacterial count of 9.0 was observed from the vegetables in this study are similar to those obtained by [11] who reported that bacterial count between 10^6-10^7cfu/g where found on watermelon, pineapple, lettuce and other vegetables. From the results obtained in table 1 lettuce had the highest total mean count of 8.26×10^4, Carrot had 6.98×10^4, cucumber had 6.88×10^4; garden egg had 6.28×10^4 while cabbage had the least total mean count of 6.26×10^4. The presence of E. coli on the vegetables analyzed is indicative of faecal contamination and other intestinal pathogen might be present. Some strains of E. coli are linked to cause of diarrhea, gastroenteritis and urinary tract infections [12]. Majority of bacteria found on the surface of plants are usually Gram- negative such as pseudomonas sppand Escherichia coli.However,bacteria can be present in low numbers as a result of the uptake of water through certain irrigation or washing procedures if these water are contaminated with human pathogens these may also be introduced. Most of the organism isolated in the study might have been introduced into these vegetables from feacal contaminated water used for irrigation. According to[13], a micro biological standard of food provides safe, sound and wholesome quality and also protect individual health.

The isolated moulds from the vegetables samples are known to produce secondary metabolites known as mycotoxins to human[14]. Asperillus species which is one of the isolated mould produces Aflotoxins which is associated with liver cancer,Fusariumtoxins which are produced by Fusariumspecies can affect the nervous system of animals and causes diarrhea in human[15].Ochratoxins which are the toxins produced by penicilliumspecies has been identified as the cause of kidney damage in human[16].The possible source of contaminating organisms associated with these products could be traced to exposure to dust and endo- spore in air,this is because the spores will colonize the vegetables samples of interest. From the result obtained from table 6, it was seen that UngwanRimi had the highest contamination of Aspergillusspp while Malali had the least contamination of Fusariumspp and Rhizopus which are known for the damages the cause of human and other animals by producing mycotoxins such as Aflatoxin, Fusarium toxic and Ochratoxic which cause several diseases in human and animals[14]. The degree of these mycotoxic producing mould present in A/Rimi could be traced to the harvesting, handling, and postharvest storage or after purchasing by the consumer. The contamination of the vegetables samples could also be as a result high moisture content of the vegetables samples.this agrees with [17]who reported that fungi colonize food with high moisture content.

Rhizopus species were isolated from most of the vegetables of interest from the various farms are among the fungi causing the group of infection referred to as Zygomycosis which agrees with [18]which stated that Rhizopus species have a wide range of distribution and can colonize all forms of food samples if conditions are favorable. Contamination of these vegetables can be reduced by using viable seeds which are free of any form of diseases for their planting. Farming also who are involved in the production and processing of these vegetables can minimize the rate of contamination by observing good farming system and good hygiene during processing.

V. Conclusion

Results obtained in the study revealed that the irrigated ready to eat vegetables are highly contaminated and have exceeded the standard microbiological limits hence influx of various kind of microorganisms into the environment. We therefore recommend that government should intervene in treatment of water used for irrigation and should also create dams as the source of water for irrigation. Farmers should be educated on the dangers of using contaminated water for irrigation of farm lands and they should also be advised on proper hygiene of vegetables before consumption and improved seed should be provided to farmers by the government.

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