



MICROORGANISMS ASSOCIATED WITH SOME SELECTED LEAFY VEGETABLES SOLD IN SOME MARKETS IN ILORIN, KWARA STATE

¹Olahan, G.S., ¹Garuba, T., ¹Adeyemi, S.B., ¹Lateef, A.A and ²Olahan, S.A.

¹Department of Plant Biology, University of Ilorin, PMB 1515, Ilorin, Kwara State, Nigeria

²Nigerian Stored Products Research Institute, PMB 1489, Ilorin, Kwara State, Nigeria

(*Correspondence address: ganiyushittuolahan@yahoo.com)

ABSTRACT

Micro-organisms associated with some commonly consumed leafy vegetables in popular markets of Ilorin, Kwara State were investigated. The leafy vegetables were *Amaranthus hybridus*, *Brassica oleraceae* and *Telfairia occidentalis*. Seven species of bacteria were isolated namely *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Streptococcus faecalis* and *Micrococcus luteus*. Six species of fungi were equally isolated from some of the same leafy vegetables namely *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifer*, *Neurospora sitophila*, *Penicillium chrysogenum* and *Mucor racemosus*. The isolates which are pathogenic might have arisen from the conditions in the fields and gardens where the leafy vegetables were grown, during transportation of the leafy vegetables to the markets or as a result of the filthy environment in the markets from where the leafy vegetables were obtained for this study. Therefore, there is a great need for increased awareness on environmental hygiene in those fields and gardens and the markets as well as sensitization of the farmers and traders on the need to adopt the modern technologies of transporting, storing and handling leafy vegetables.

Keywords: Environmental hygiene, Leafy vegetables, Bacteria, Fungi, Sensitization.

INTRODUCTION

Leafy vegetables are plant leaves eaten as a vegetable, sometimes accompanied by tender petioles and shoots. They most often come from short-lived herbaceous plants such as lettuce and spinach. They contain many typical plant nutrients, and are the primary food class that interacts significantly with warfarin, the anticoagulant pharmaceutical. They are low in calories, low in fat, high in protein per calorie, high in dietary fibre, high in iron and calcium, and very high in phytochemicals such as vitamin C, lutein, carotenoids, folate, magnesium as well as vitamin K (Kochlar, 2009). They also provide varying amounts of chlorophyll and contain lots of water. They include salad greens, spinach, collards, kale, lettuce, turnip greens, beet greens, cabbage, pumpkin, *Amaranthus*, etc. Consumption of fruit and vegetable products has dramatically increased in the U. S. by more than 30% during the past decades. It is also estimated that about 20% of all fruits and vegetables produced is lost each year due to spoilage (Barth *et al.*, 2009). Most of the fresh vegetables are lost annually due to spoilage caused by microorganisms that are initially observed on their surface. Vectors for disseminating these microbes include soil particles, airborne spores, and irrigation water. Most bacteria and fungi that arrive on the developing vegetable either are completely benign to the crop's health or, in many instances, provide a natural biological barrier to infestation by the subset of microorganisms responsible for crop damage (Janisiewicz and Korsten, 2002; Andrews and Harris, 2000). Spoilage microbes can be introduced to the vegetables during growth in

the field, during harvesting and postharvest handling, or during storage and distribution (Barth *et al.*, 2009). The aim of this study was to investigate the microorganisms associated with some commonly consumed leafy vegetables in popular markets of Ilorin, Kwara State while the objectives were to determine the pH values, percentage moisture content and percentage ash content of samples of the leafy vegetables used in the study. The study was justified by the fact that the samples of leafy vegetables used are largely bought for consumption from the chosen markets, which are very large and popular.

MATERIALS AND METHODS

Collection and identification of vegetable samples

Cabbage (*Brassica oleraceae* var. capitata), fluted pumpkin (*Telfairia occidentalis*) and Jute (*Amaranthus hybridus*) were obtained from Ipata, Ojatonun and Challenge markets all within Ilorin metropolis, Kwara State on 12th May, 2012. The markets are located about 5km away from each other and the leafy vegetable sellers are well patronaged daily by consumers of the leafy vegetables. The leafy vegetables were stored in separately labeled sterilized polythene bags and taken to the Plant Biology laboratory, University of Ilorin. Their scientific names were obtained from the Curator at the Herbarium of the Department of Plant Biology, University of Ilorin. Each of the samples was surface-sterilized separately using 70% ethanol to swab the surfaces of the samples and rinsed in 3 changes of sterile distilled water. The sterilized samples were stored separately in a refrigerator prior to use.



Physico-chemical analysis of the leafy vegetable samples

Procedures for determination of moisture contents according to AOAC (2000) as adopted by Musa *et al.* (2010) were followed. Air-dried samples of each of the leafy vegetables were ground to pass through a one millimeter screen in a Christy and Norris 20cm Laboratory Hammer Mill Machine (London) and kept separately. A moisture dish was dried in a Gravity Convection Oven regulated at 103°C for 30 minutes, transferred into a desiccator to cool and weighed. Five

grams (5.0g) of one of the leafy vegetable powder was put in the moisture dish and re-weighed. The dish with the sample was then placed in the oven for hours at 103°C. The dish with the dry sample was transferred into desiccator to cool and re-weighed. The above procedures were done in replicates and repeated for each of the remaining powders of the leafy vegetables used in this study. Their respective percentage moisture content was determined using the following calculation:

$$\% \text{ Moisture content} = \frac{\text{Mass of dish + Wet Sample} - \text{Mass of dish + leafy vegetable powder}}{\text{Mass of dish + leafy vegetable powder}} \times 100$$

The percentage ash content of each of the leafy vegetables was determined following the methods of Okalebo *et al.* (2002) by incinerating 5.0g of each of the powders produced above in a Muffle Furnace at

600°C for three hours. The ashes were cooled in desiccators and then weighed. These procedures were done in replicate, and their respective percentage ash content was estimated using the following calculation:

$$\text{Ash content (\%)} = \frac{\text{Mass of Ash}}{\text{Mass of leafy vegetable powder}} \times 100$$

The pH values of each of the leafy vegetables were measured in three replicates using the methods of Henry and Boyd (1998). Twenty grams of each of the leafy vegetable powders prepared earlier was dissolved in 20ml of distilled water inside three 250ml conical flasks. Each suspension was constantly stirred for 20 minutes to enable each of them to reach equilibrium (Henry and Boyd, 1998). Their pH values were determined using the Searchtech Instrument PHS – 3c Model pH meter which had been pre-standardized with buffers 4, 7 and 9.

Microbiological analysis of the leafy vegetable samples

The micro-organisms associated with the leafy vegetables were isolated following the procedures of Okaisabor (1968). Two sets of small sections of each of the sterilized samples were plated on separate potato dextrose agar (PDA) medium to isolate fungi and nutrient agar (NA) medium for the isolation of bacteria in 9.0 cm Oswald Petri dishes. The plates were incubated at 25± 2°C (for 24 – 96 hours for fungal growth) and 37°C (for 24 – 48 hours for bacterial growth). Developing cultures on each of the plates were subcultured on respective fresh media plates until pure cultures of fungi and bacteria were obtained. The pure cultures of fungal and bacterial isolates were then stored on agar slants in McCartney bottles and stored in a refrigerator prior to use. The fungal isolates were subjected to microscopic examination and identification was done with reference to Barnett and Hunter (2010) as well as Campell and Stewart (1980). The bacterial isolates were identified based on the result of biochemical tests carried out on them following the methods of Fawole and Oso (2004).

RESULTS AND DISCUSSION

The moisture content of *Brassica oleraceae* obtained from Ipata market was found to be numerically highest (84.51%) and *Telfairia occidentalis* obtained from the same market recorded the lowest (73.70%). The percentage ash content in *Amaranthus hybridus* was estimated to be 0.45% (Ojatuntun) and the value was precisely the same with the value recorded for *Amaranthus hybridus* from Ipata. However, all the three samples from different markets had pH values lower than 7.00 but greater than 4.00. Thus, they were slightly acidic as shown in Table 1. Total of 5 fungi isolates were recorded from all the samples from three markets in Ilorin. *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifer*, *Neurospora sitophila*, *Penicillium chrysogenum* and *Mucor racemosus* were detected as shown in Table 2. *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium chrysogenum* *Mucor racemosus* were present in all the samples of the leafy vegetables obtained from the three markets considered in this study. *Aspergillus flavus* was absent in *Amaranthus hybridus* and *Brassica oleraceae* samples from Challenge Market but present in the same samples obtained from Ipata and Ojatuntun Markets. *Neurospora sitophila* was present in *Amaranthus hybridus* obtained from Ipata market but absent in the same samples obtained from Challenge and Ojatuntun markets. *Mucor racemosus* was present in *Amaranthus hybridus* and *Telfairia occidentalis* obtained from Challenge market but absent in the same leafy vegetable and the other leafy vegetable sample obtained from Ipata and Ojatuntun markets (Table 2).



Table 1: Physico-chemical properties of the leafy vegetables collected from three markets in Ilorin

Locations/Vegetable - Samples	% Moisture content	% Ash content	pH Values
Challenge:			
<i>Telfairia occidentalis</i>	77.14	0.43	5.41
<i>Amaranthus hybridis</i>	75.94	0.40	6.01
<i>Brassica oleraceae</i>	79.73	0.50	5.65
Ipata:			
<i>Telfairia occidentalis</i>	73.70	0.40	5.70
<i>Amaranthus hybridis</i>	78.91	0.45	5.77
<i>Brassica oleraceae</i>	84.51	0.80	4.75
Ojatuntun:			
<i>Telfairia occidentalis</i>	74.55	0.35	5.06
<i>Amaranthus hybridis</i>	78.74	0.45	5.75
<i>Brassica oleraceae</i>	81.27	0.75	5.02

*Each value is a mean of 3 replicates of each of the plant materials

Table 2: Occurrence of fungal isolates in the leafy vegetables from selected markets in Ilorin

Locations/Vegetable - Samples	Fungal isolates					
	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Rhizopus stolonifer</i>	<i>Neurospora sitophila</i>	<i>Penicillium chrysogenum</i>	<i>Mucor racemosus</i>
Challenge:						
<i>Telfairia occidentalis</i>	+	-	+	-	+	+
<i>Amaranthus hybridis</i>	+	-	+	-	+	+
<i>Brassica oleraceae</i>	+	-	+	-	+	-
	3/3	0/3	3/3	0/3	3/3	2/3
Ipata:						
<i>Telfairia occidentalis</i>	+	-	+	-	+	-
<i>Amaranthus hybridis</i>	+	+	+	+	+	-
<i>Brassica oleraceae</i>	+	+	+	-	+	-
	3/3	2/3	3/3	1/3	3/3	0/3
Ojatuntun:						
<i>Telfairia occidentalis</i>	+	-	+	-	+	-
<i>Amaranthus hybridis</i>	+	+	+	-	+	-
<i>Brassica oleraceae</i>	+	+	+	-	+	-
	3/3	2/3	3/3	0/3	3/3	0/3
TOTAL	9/9	4/9	9/9	1/9	9/9	2/9

Keys:

(+) = Present

(-) = Absent

A. niger = *R. stolonifer* = *P. chrysogem* > *A. flavus* > *M. racemosus* > *N. silophyla* in terms of occurrence in all the markets

Seven (7) bacterial isolates belonging to different genera were recovered from samples of the leafy vegetables obtained from the three popular markets in Ilorin metropolis, though they were not uniformly present. They were designated B₁ to B₇ and later identified based on their colonial morphology, results of cellular and biochemical characteristics such as Gram's staining, motility test, spore staining, starch hydrolysis, catalase test, coagulase test, capsule stain, indole test, oxidase test, oxygen relationship, urease test, Voges Proskauer's test, citrate utilization test, methyl red test and sugar fermentation test carried out on the bacterial isolate following the procedures of Fawole and Oso (2007). Isolate B₁ was identified as *Escherichia coli*, B₂ was *Staphylococcus aureus*, B₃ was *Bacillus subtilis*, B₄ was *Pseudomonas aeruginosa*, B₅

was *Enterobacter aerogenes*, B₆ was *Streptococcus faecalis* while B₇ was *Micrococcus luteus* (Table 3). *Escherichia coli* and *Pseudomonas aeruginosa* were present only in the samples of *Amaranthus hybridis* and *Telfairia occidentalis* obtained from Ipata market and absent in samples of *Brassica oleraceae*. These bacterial isolates were absent from all the samples of the leafy vegetables obtained from Ojatuntun and Challenge markets. This may be as a result of the filthy environment of Ipata market compared to the other 2 markets. *Staphylococcus aureus*, *Streptococcus faecalis* and *Micrococcus luteus* were present in all the samples of the leafy vegetables obtained from the three markets under survey (Table 3).



Bacillus subtilis was present in the samples of *Amaranthus hybridus* and *Telfairia occidentalis* obtained from Challenge and Ojatuntun markets but absent from the same leafy vegetables obtained from Ipata market. *Enterobacter aerogenes* was present in

the samples of *Amaranthus hybridus* and *Brassica oleraceae* obtained from Ipata and Ojatuntun markets, but absent from the same leafy vegetables obtained from Challenge market (Table 3)

Table 3: Occurrence of bacterial isolates in the leafy vegetables from selected markets in Ilorin

Locations/Veg etable Samples	Bacterial isolates						
	<i>E. coli</i>	<i>S. aureus</i>	<i>Bacillus subtilis</i>	<i>Enteroba. aerogenes</i>	<i>Strept. faecalis</i>	<i>Pseudo. aeruginosa</i>	<i>Microc. luteus</i>
Challenge:							
<i>Telfairia</i>	-	+	+	-	+	-	+
<i>occidentalis</i>	-	+	+	-	+	-	+
<i>Amaranthus hybridus</i>	-	+	+	-	+	0/3	3/3
<i>Brassica oleraceae</i>	0/3	3/3	2/3	0/3	3/3		
Ipata:							
<i>Telfairia</i>	+	+	-	-	+	-	+
<i>occidentalis</i>	+	+	-	+	+	+	+
<i>Amaranthus hybridus</i>	+	+	-	+	+	1/3	3/3
<i>Brassica oleraceae</i>	2/3	3/3	0/3	2/3	3/3		
Ojatuntun:							
<i>Telfairia</i>	-	+	+	-	+	-	+
<i>occidentalis</i>	-	+	+	+	+	+	+
<i>Amaranthus hybridus</i>	-	+	+	+	+	1/3	3/3
<i>Brassica oleraceae</i>	0/3	3/3	2/3	2/3	3/3	2/9	9/9
TOTAL	2/9	9/9	4/9	4/9	9/9		

Keys: (+) = Present (-) = Absent
E. coli = *P. aeruginosa*, < *B. subtilis* = *E. aerogenes* < *S. aureus* = *S. faecalis* = *M. luteus* in terms of occurrence in all the markets

The presence of these micro-organisms in the leafy vegetables was a confirmation of the availability of the nutrients required by them such as protein, minerals, amino acids, lipids and vitamins as reported by Ihekoronye and Ngoddy (1985). Some of these micro-organisms may also be contaminants from the atmosphere or surrounding of the environment from which the leafy vegetables were obtained or from fields and gardens where they were grown. It was found that samples of the leafy vegetables obtained from Ipata and Ojatuntun markets had the highest presence of the bacterial and fungal isolates while samples of leafy vegetables obtained from Challenge market had the lowest bacterial and fungal presence. These may be as a result of the filthy nature of the Ipata and Ojatuntun markets compared to the neat environment of Challenge market. The Ipata and Ojatuntun markets are cosmopolitan markets while the Challenge market is modern. According to Barth et al (2009), the kind and number of micro-organisms present in a food item is influenced by the type and extent of contamination, previous opportunities for growth and pre-treatment which the food item had received. All the fungal and bacterial isolates agreed with the results of Ben - Uwabor (2004), Olatolu

(1998) and Bamberg et al (1996). They cause a great loss in the yield and nutritional values of vegetables. *Rhizopus stolonifer* causes soft-rot in vegetables (Gilman, 1959). *Aspergillus* species have been reported to cause diseases such as pulmonary aspergillosis, pneumonia, extrinsic asthma and allergic aveolitis in susceptible individuals (Lacey, 1975; Austwick, 1965; Gilman, 1959). From the above, it is certain that if these contaminated leafy vegetables are eaten without proper cooking by susceptible humans, the life of such people will be at the risk of contracting these diseases. *Aspergillus flavus* produce aflatoxins and cycloplazonic acid (CPA). Aflatoxins are mutagenic and carcinogenic to man. Aflatoxins are responsible for damaging up to 25% of the world's food crops, resulting in large economic losses in developed countries and human and animal disease in under-developed ones (Agrios, 2005). There is also a great risk of contracting gastro - intestinal infections through the consumption of these leafy vegetables sold in the 3 markets under study. Food poisoning is also likely to be of common occurrence if the *Staphylococcus aureus* infested leafy vegetables from the markets under study are consumed.



CONCLUSION

The leafy vegetables studied had seven bacterial isolates and six fungal isolates, which are pathogenic to animals and man.

RECOMMENDATIONS

Leafy vegetable sellers in the markets under study should be sensitized on the need to adopt modern

handling and storage techniques developed by the Nigerian Stored Products Research Institute (NSPRI), so as to prolong the shelf-life of their products as well as make them to be free from contamination by micro-organisms. These technologies include storage at low temperatures, storage in bulk at ambient temperature and storage in vegetable baskets.

REFERENCES

- Agrios, G. N. (2005): *Plant Pathology*. Fifth edition. Elsevier Academic Press, U.S.A. 922pp.
- Andrews, J.H. and Harris, R.F. (2000): The Ecology and Biogeography of Microorganisms on Plant Surfaces. *Annual Review of Phytopathology* 38:145-180.
- Austwick, P.K.C. (1965): *Pathogenicity of Aspergillus spp.* In: Kaper, K.B. and Fennel, D.I. *The Genus Aspergillus*. Williams and Wilkins, Baltimore, M.D. pp82 - 126.
- Bamburg, J.R., Strong, F.M. and Smalley, E.B. (1996): Toxin from mould cereals. *Journal in Agricultural Chemistry* 17: 443 - 450.
- Barnett, H. L. and Hunter, B. B. (2010): *Illustrated Genera of Imperfect Fungi*. Fourth Edition. The American Phytopathological Society, St. Paul, Minnesota. 94p.
- Barth, M., Hankinson, T.R., Zhuang, H. and Breidt, F. (2009): *Microbial Spoilage of Fruits and Vegetables*. In: Sperber, W.H. and Doyle, M.P. (eds.). *Compendium of the Microbiological Spoilage of Foods and Beverages*. Food Microbiology and Food Safety. Springer Science Business media, LLC. pp 135-183.
- Ben-Uwabor (2004): *Isolation of Fungi associated With Cabbage and Lettuce*. M.Sc. Plant Pathology project Report, University of Ilorin, Kwara State, Nigeria.
- Campbell, M.C. and Stewart, J.L. (1980): *The Medical Mycology Handbook*. A Wiley Medical Publication. John Wiley and Sons. Pp229-314.
- Fawole, M.O. and Osho, B.A. (2004): *Laboratory Manuals of Microbiology*. Spectrum Books Ltd., Ibadan, Nigeria.
- Gilman, J.C. (1959): *A manual of the Soil Fungi*. Iowa State University press, Ames, Iowa.
- Henry, D.F. and Boyd, G.E. (1988): *Soil Fertility*. John Wiley and Sons, New York. 212pp.
- Ihekoronye A.I. and Ngoddy P.O. (1985): *Integrated food science and tech for the tropics*. 1st edition., Macmillan Publisher Ltd, London.
- Janisiewicz, W.J. and Korsten, L. (2002): Biological Control of Postharvest Diseases of Fruits. *Annual Review of Phytopathology* 40:411-441.
- Kochlar, S.L. (2009): *Economic Botany in The Tropics*. 3rd. Edition. Pp224 - 268.
- Lacey, J.P. (1975): Hazards to animals and man from micro-organisms in fodder and grains. *Trans. Brazilian Mycology Society* 65:171 - 184.
- Musa, A.K., Kalejaiye, D.M, Ismail, L.E. and Oyerinde, A.A. (2010): Proximate composition of selected groundnut varieties and their susceptibility to *Trogoderma granarium* Everts attack. *Journal of Stored Products and Post Harvest Research* 1(2):13-17.
- Okaisabor, E.K. (1968): An active Fungi parasite of *Phytophthora palmivora*. *PANS(B)* 14:353.
- Okalebo, J.R., Gathua, K.W. and Woomer, P.L. (2002): *Laboratory methods of soil and plant analysis: A working manual*. Second edition). Nairobi, Kenya.
- Okigbo, R. and Emoghene, A. (2000): Root and bottom rot of orange trees in South Western Nigeria. *Fruits* 55(5):309 - 314.
- Olaolu, J. (1998): *Mycoflora Associated with grains of Sorghum bicolor (L.) Moench cultivated in I'orin, Nigeria, and their chemical control using two conventional seed dressers and dry neem seed powder*. M.Sc. Project Report, University of Ilorin, Kwara State, Nigeria. Pp74-82.