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Microbiological Quality and Antibiotic Susceptibility Profile of Microorganisms Associated with Stored Vegetables in Port Harcourt

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Vegetables are good sources of nutrients such as minerals, oil, vitamins and carbohydrates and are used in preparing different delicacies such as soups and salads. This study was aimed at investigating the microbiological quality and antibiotic resistance pattern of microorganisms associated with stored vegetables in raffia baskets.
Methodology: The vegetables were obtained from the Nigerian Stored Products Research institute (NSPRI) farm in Port Harcourt. Ten grams (10 g) each of fresh vegetables were homogenized differently in 90ml of sterile diluent. Aliquot (0.1 ml) of 10^{-3} and 10^{-5} dilutions of each vegetable sample was plated on nutrient agar plates and incubated at 37ºC for 24hours. Identified isolates were standardized using the 0.5 McFarland standard. This was done by transferring colonies of the test isolates into sterile 4 mL normal saline and comparing the turbidity of the isolate in the test tubes with the already prepared 0.5 McFarland. The disc diffusion method was used in determining the susceptibility pattern of the microorganisms against the antibiotics. In this method, the standardized inoculums were seeded aseptically on freshly prepared Mueller Hinton agar plates. Whatman discs which have been impregnated with different concentrations of the antibiotics were placed on the seeded plates and incubated at 37ºC for 18-24 hours.

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Results: Zones of clearance or inhibition on plates were recorded. The genera *Bacillus, Pseudomonas, Enterococcus, Enterobacter, Bordetella, Staphylococcus, Myroides, Escherichia, Serratia, Micrococcus* and *Acetobacter* were identified as predominant microbes from the vegetables.
Conclusion: Despite the high level of resistance to the antibiotics, Ciprofloxacin and Ofloxacin were the most effective and preferred drugs of choice for treatment of infections arising from the consumption of these vegetables. Adequate heating and blanching of vegetables is required at all times to prevent food poisoning.

Keywords: Vegetables; storage; microorganisms; antibiotic profile; temperature; humidity.

1. INTRODUCTION

Fresh Vegetables are often called protective foods and are needed in the makeup of a healthy meal [1] because of the various health benefits they offer [2]. They are important sources of minerals, vitamins, fiber, carbohydrates, oils and other micronutrients required in daily meals [3]. In Nigeria, vegetables are chewed crude or blanched so as to retain its organoleptic properties and in most cases responsible for food– borne infections [4]. In the process of planting and harvesting or storage, disease-causing microorganisms may contaminate these vegetables through contact with natural wastes such as fecal matter, sewage and other organic wastes which are used as humus usually obtained from compost, or untreated water or surface waters used to improve water activity (a_w) and the soil quality [5]. The water sprinkled on the vegetables to keep them fresh may also be a source of microbial tainting [6]. In the village settings and some urban centers, vegetables are stored in covered baskets made with bamboo sticks and wrapped with jute bags. The wetting of the basket and evaporation from the basket gives a cooling effect to the inside of the basket making it conducive for survival of the leaves for a longer period of time compared with when they are left in the open [7,8]. However, spoilage occurs when they are left for longer periods of time beyond the storage capacity. Preservation of vegetables is of importance as it enhances its shelf life which makes them available whenever it is needed, because these vegetables are perishable foods. Several microorganisms isolated from vegetables have been reported to be resistant to several antibiotics. Daniel *et al* [9] reported that the resistance of pathogenic microorganisms isolated from foods poses threat to safety of foods and the consumers. A study on microorganisms isolated from fluted pumpkin recorded 100% resistance of *Pseudomonas vulgaris* to Chloramphenicol, Gentamicin and Trimethoprim while *Pseudomonas agglomera P. fluorescens, Bacillus cereus, B. subtilis, Kebsiela pneumoniae, Staphylococcus aureus, S. epidermidis and E. aerogenes* also exhibited varied resistance pattern to different antibiotics [10]. There has been paucity of information on the susceptibility pattern of microbes associated with stored leafy vegetables from markets in Port Harcourt. It is therefore, important to evaluate the microbial quality and antibiotic resistance pattern of microorganisms associated with stored vegetables as this will provide public health data on these edible leaves to be able to handle food–borne infections

2. MATERIALS AND METHODS

2.1 Sample Collection

Five (5) different vegetables including pumpkin leaf, bitter leaf, scent leaf, water leaf and okazi leaf were obtained from Nigerian Stored Products Research institute (NSPRI) farm in Port Harcourt and transported to the Microbiology laboratory of the Rivers State University for analyses.

2.2 Preparation of Samples for Storage

Raffia baskets were treated with alcohol for 24 hours under aseptic conditions before the vegetables were placed into the baskets for storage. One litre of water was sprinkled on the vegetables twice daily. Temperature and humidity were taken periodically every six (6) hours to determine the change in temperature due to heat generated and humidity changes until spoilage occurred. Another set of vegetables were kept in open air as control and monitored for changes, this served as control. Similar readings were obtained.

Ten grams (10 g) of each vegetable was homogenized in 90ml of sterile diluent after which the homogenized samples were diluted serially to 10^{-6} [11].

2.3 Inoculation and Enumeration of Colonies

About 0.1 ml of 10^{-3} to 10^{-5} dilutions were transferred to freshly prepared Nutrient agar plates in duplicates with the aid of sterile 1ml pipettes. Plates were inoculated by spreading the inoculum using sterile bent glass rod and incubated at 37ºC for 24 hours. After incubation, plates were observed for growth. Discrete colonies were counted as total heterotrophic bacteria on plates.

2.4 Maintenance of Pure Culture

Discrete bacterial colonies that grew on the Nutrient Agar plates were sub-cultured using streaked plate method by transferring each colony of same morphology onto freshly prepared NA plates under strict aseptic conditions. Bacterial isolates were purified by repeated sub-culture onto prepared Nutrient agar plates. Pure cultures were transferred onto Nutrient agar slants and incubated at 37ºC for 24 hours and then preserved in the refrigerator at 4ºC for further analyses [12].

2.5 Antimicrobial Susceptibility Test

The antibiotic sensitivity of the bacterial isolates was determined using the disc diffusion method [13]. Multiple antibiotic sensitivity testing was also carried out using the method adopted by [14]. In this method, standardized inoculums of 24-hour cultures were spread vertically and horizontally on Mueller-Hinton agar plates using sterile swab sticks. The plates were allowed to dry for 5 minutes before placing the antibiotic discs at the centre using sterile forceps. The plates were incubated at 37ºC for 24h and the diameter of zones of inhibition was measured in millimetre. Result was interpreted as sensitive, intermediate or resistant, based on the Clinical and Laboratory Standard institutes [15].

3. RESULTS AND DISCUSSION

In this study, a total of three hundred and fifty-four (354) bacterial isolates were recorded (Table 1) The result revealed that the predominant bacterial genera were *Bacillus, Pseudomonas, Enterococcus, Enterobacter, Bordetella, Staphylococcus, Myroides, Escherichia, Serratia, Micrococcus and Acetobacter*. *Bacillus* sp occurred in all the vegetables all through the period of storage; while *Enterococcus faecalis, Acetobacter orientalis, Bordetalla pertussis, Myroides xuawensis* and *Bacillus flexu*s were isolated from the stored vegetable on the third day in most of the vegetables. Table 1 shows that *Bordetella pertussis* and *Bacillus flexus* were isolated in all the five vegetables. Scent leaf had the highest occurrence of *Bordetella* sp 38% followed by Bitter leaf 22% while water leaf had the least occurrence with 2%. *Enterobacter xiangfangensis* were isolated in all vegetables except for scent leaf, with its highest occurrence in water leaf 16% and Bitter leaf and Pumpkin leaf 12% respectively. *Enterobacter cloaca* occurred in all the vegetables but had 17% occurrence in okazi leaf. *Bacillus flexus* was predominant in pumpkin and scent leaves. Table 1 also showed that scent leaf had the highest microbial population of 94%, bitter leaf and pumpkin had 73%, while 56% and 58% occurrence were recorded for water leaf and okazi respectively. The bacterial isolates identified in this study agrees with findings of Mandrell et al. [16], on similar fresh vegetables.

Microorganisms belonging to the genus *Bacillus* are spore forming bacteria and often heat-resistant. They can be found in different environments due to their ubiquitous nature and presence of endospores. They are able to cause opportunistic infections such as endocarditis, bacteremia and wound abscesses [17], *Bacillus subtilis* causes mediastinitis and bacteremia [18]. Organisms belonging to the genus *Myroides* are able to cause skin infections in patients with diabetes as well as urinary tract infections [19]. *Bordetella pertussis* is the causative agent of whooping cough while *Enterobacter* causes meningitis, pneumonia, bacteremia as well as urinary tract infection. *Enterococcus faecalis* are emerging as hospital pathogens and are mostly found in the gut and may cause nosocomial infections or as food-borne pathogens. They are able to produce cytolysin (streptolysin) that affects the gut cause multi-drug resistance [20].

There was no relationship between the temperature and humidity of stored waterleaf, okazi and pumpkin indicating that as the temperature in the basket increased, the relative humidity decreased and vice versa while it was observed that scent leaf and bitter leaf had a correlation, indicating that when temperature increases, a corresponding increase in humidity was recorded. Also, relationship between temperature and time for water leaf and bitter leaf had no correlation indicating that the vegetables remained in the baskets, the

temperature decreased daily. The correlation between temperature and time for okazi leaf, pumpkin leaf and scent leaf showed that the temperature increased daily in the basket. Correlation of humidity and time showed that water leaf had a positive correlation so the relative humidity in the basket increased daily. That of okazi leaf, pumpkin leaf, scent leaf and bitter leaf reduced daily.

Fig. 1 shows the relationship between temperature and time for stored water leaf sample. The mean temperature value was 26.96 while the standard deviation is 1.60. The correlation between temperature and time was 0.78 (R^2 = 0.78).

Fig. 2 shows the relationship between relative humidity and time for stored water leaf samples. The mean relative humidity value had 93.67 while the standard deviation is 2.24. The correlation between relative humidity and time was 0.77 while the correlation between temperature and relative humidity was -0.89336 (R^2 = -0.89336).

Fig. 3 shows the relationship between temperature and time for stored okazi leaf sample. The mean temperature value was 26.15 while the standard deviation was 0.87. The correlation between temperature and time was 0.513509058.

Fig. 4 shows the relationship between relative humidity and time for stored okazi leaf sample. The mean relative humidity value was 97.97 while the standard deviation was 0.83. The correlation between relative humidity and time was 0.491.

Fig. 5 shows the relationship between temperature and time for stored pumpkin leaf. The mean temperature value was 26.51 and the standard deviation was 0.99. The correlation between temperature and time was 0.28 (R^2 =0.28).

Table 1. Percentage distribution of the bacterial Isolates in the Vegetables

Isolates	Water leaf	Scent leaf	Okazi leaf	Bitter leaf	Pumpkin leaf	Total number	Percentage (%)
Bordetella pertussis	2	38	5	22	3	70	19.77
Enterobacter xiangfangensis	16	0	3	12	12	43	12.15
Enterobacter cloacae	2	17	0	16	9	44	12.42
Myroides xuanwuensis	0	6	14	4	11	35	9.89
Acetobacter orientalis	15	9	11	10	0	35	9.89
Enterobacterfaecalis	6	0	4	7	15	32	9.04
Bacillus subtilis	18	13	12	0	5	48	13.56
Baccillus flexus	7	11	9	2	18	47	13.28
Total	56	94	58	73	73	354	100

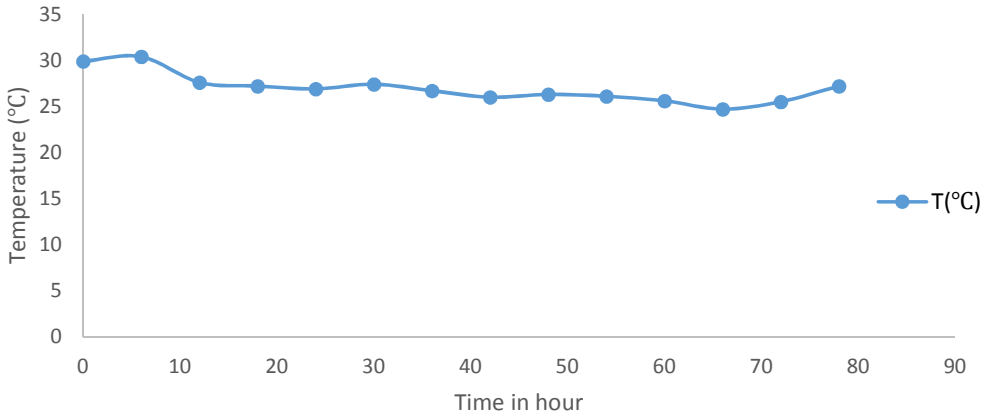

Fig. 1. The relationship between temperature and time for stored Water leaf samples

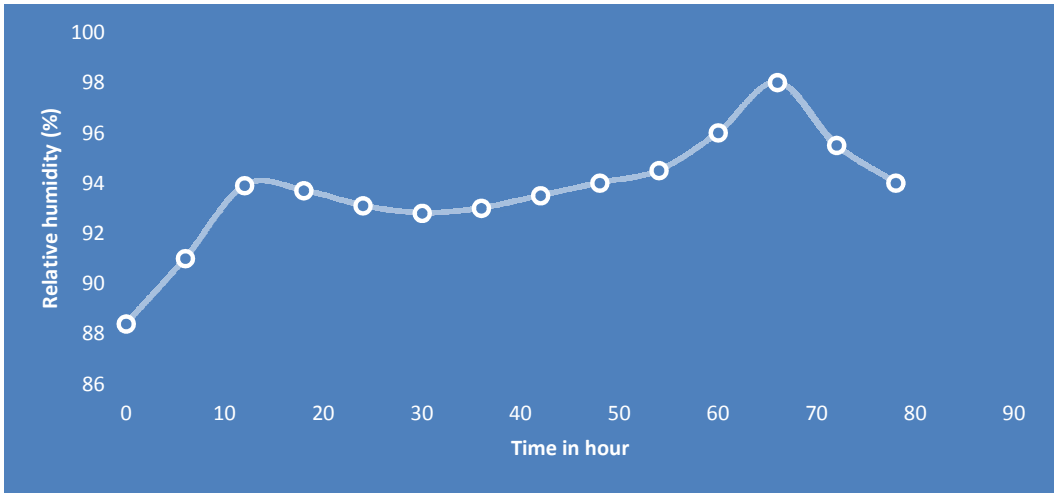

Fig. 2. The relationship between relative humidity and time for stored water leaf

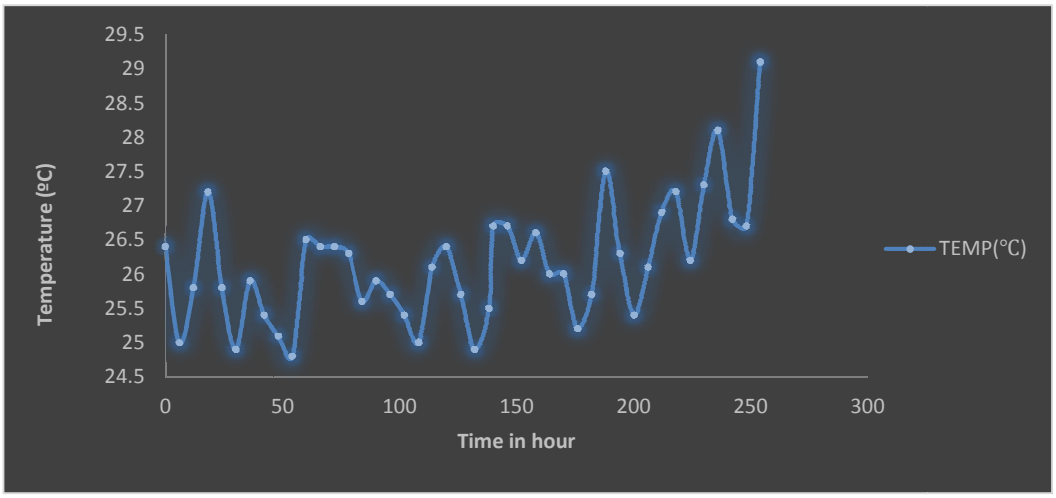

Fig. 3. The relationship between temperature and time for stored okazi sample

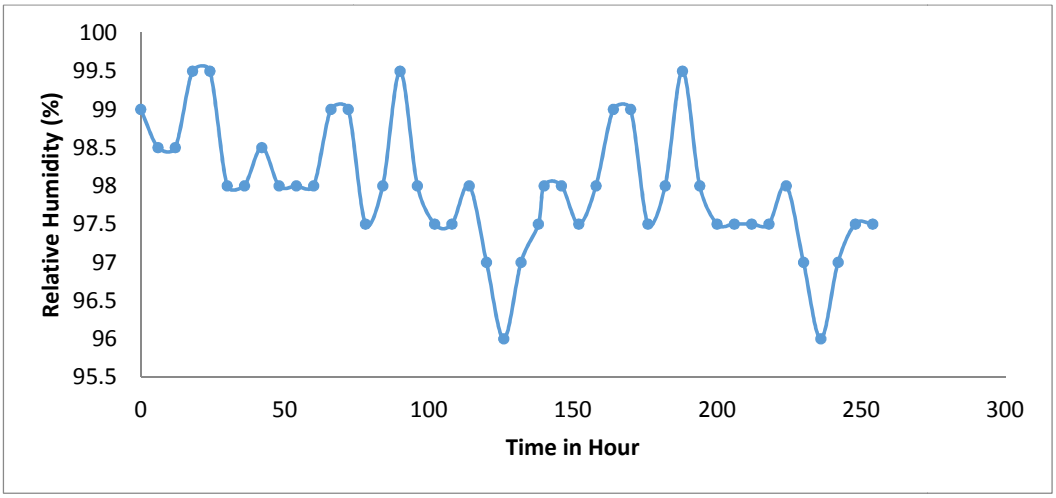

Fig. 4. The relationship between relative humidity and time for stored okazi leaf

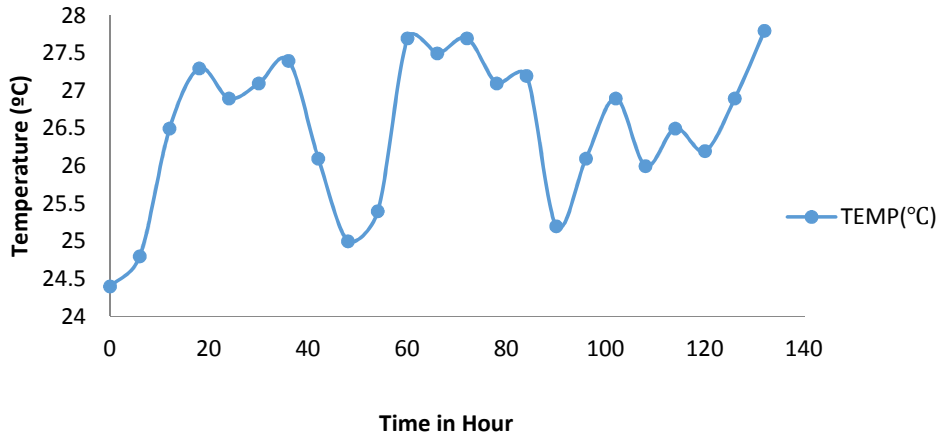

Fig. 5. The relationship between temperature and time for stored pumpkin leaf
Mean SD = 26.51±0.99. Relationship between time and temperature R^2 = 0.28

Fig. 6 shows the relationship between relative humidity and time for stored pumpkin leaf. The mean relative humidity value was 96.39 and the standard deviation was 1.48. The correlation between time and relative humidity was recorded as – 0.59 (R^2 = -0.59) and the correlation between temperature and relative humidity was -0.88 (R^2 = - 0.88) while Fig. 7 shows the relationship between temperature and time for stored scent leaf. The mean temperature value was 26.78 and standard deviation was 1.172. The correlation between temperature and time was 0.064 (R^2 = 0.064).

Fig. 8 shows the relationship between relative humidity and time. The mean relative humidity value was 94.38 and the standard deviation recorded 2.420. The correlation between and time was 0.16 (R^2 = 0.16) while the correlation between relative humidity and temp was -0.89 (R^2 = -0. 89).

Fig. 9 shows the relationship between temperature and time for stored bitter leaf. The mean temperature value reads 26.46 and standard deviation was 0.76. The correlation between temperature and time was 0.24 (R^2 = 0.24).

Fig. 10 shows the relationship between the relative humidity and time for stored bitter leaf. The mean relative humidity value was 98.09 and

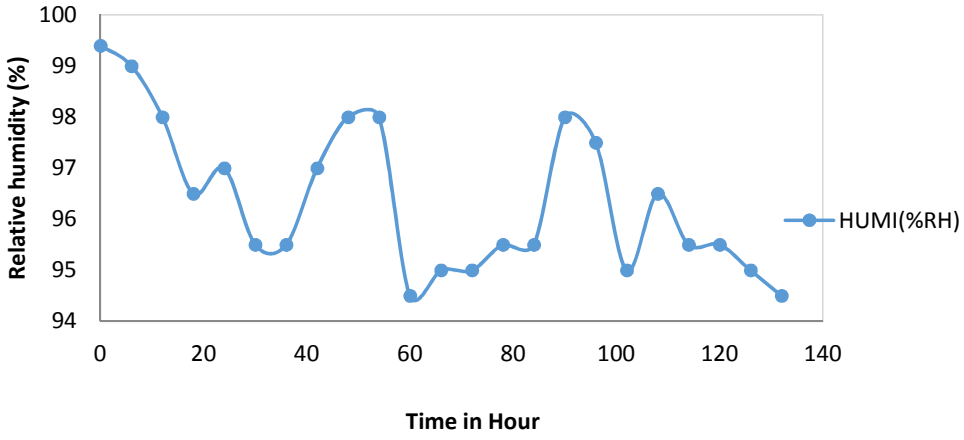

Fig. 6. The relationship between relative humidity and time for stored pumpkin leaf

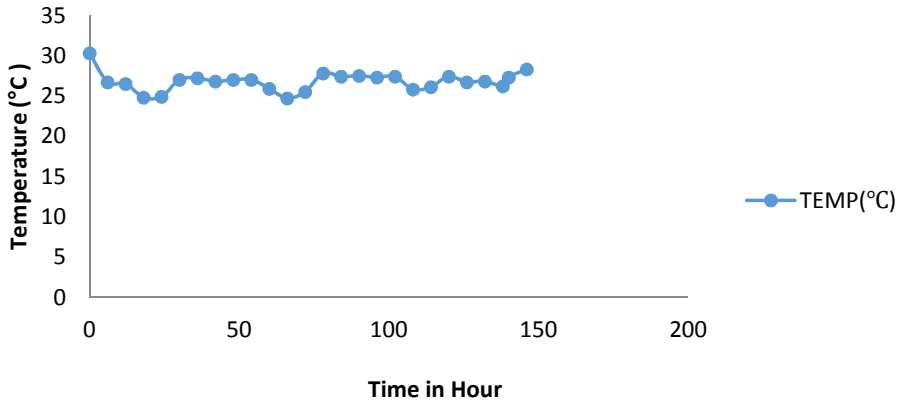

Fig. 7. The relationship between temperature and time of stored Scent leaf

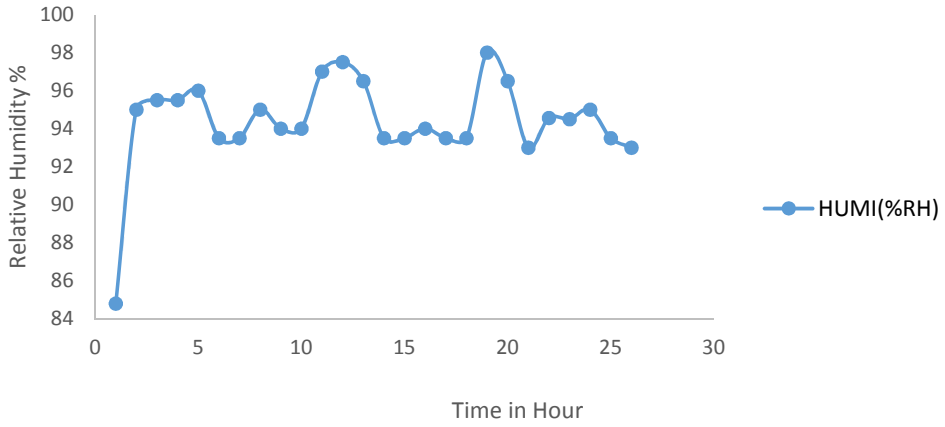

Fig. 8. The relationship between relative humidity and time for Scent leaf

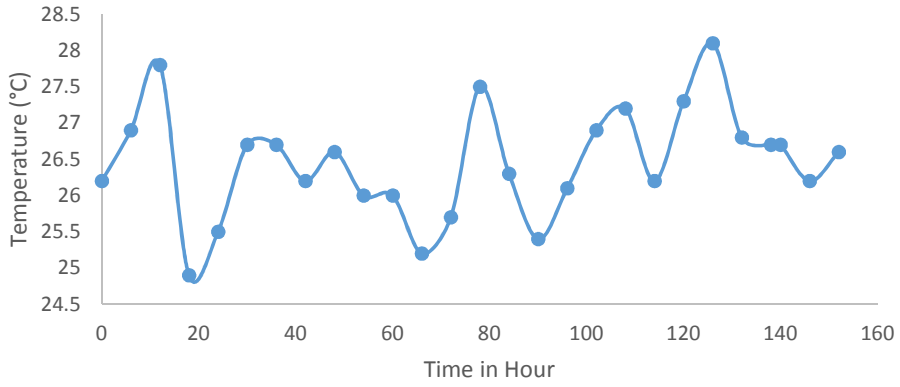

Fig. 9. The relationship between temperature and time for stored Bitter leaf

the standard deviation was 0.82. The correlation between relative humidity and time was -0.62 (R^2 = 0.62) while the correlation between relative humidity and temperature was -0.77804 (R^2 = -0.77804).

According to Prescott et al. [21], environmental factors including temperature and humidity could influence the growth of microorganisms. This influence could either be positive in terms of increase in microbial population or it could

be negative by causing a reduction in microbial population. Thus, these factors could have caused the variations in microbial genera as well as microbial load recorded in this study.

The upsurge of resistance of microorganisms to commonly used antibiotics is an emerging and global problem or trend causing serious concerns [22]. The result for the antimicrobial susceptibility showed that all the bacterial genera exhibited multi-drug resistance to the antibiotics (Figs. 11 and 12). Previous studies have reported the resistance of microorganisms to commonly used antibiotics possibly due to indiscriminate use of antibiotics, alteration of drug binding or target sites as well as the transfer of resistant genes [20,13,23,24]. Similar findings on the resistance of some bacteria to Augmentin, Amoxicillin and Gentamycin have been reported earlier on the incidence of antibiotic resistance among bacterial species [25,23,26].

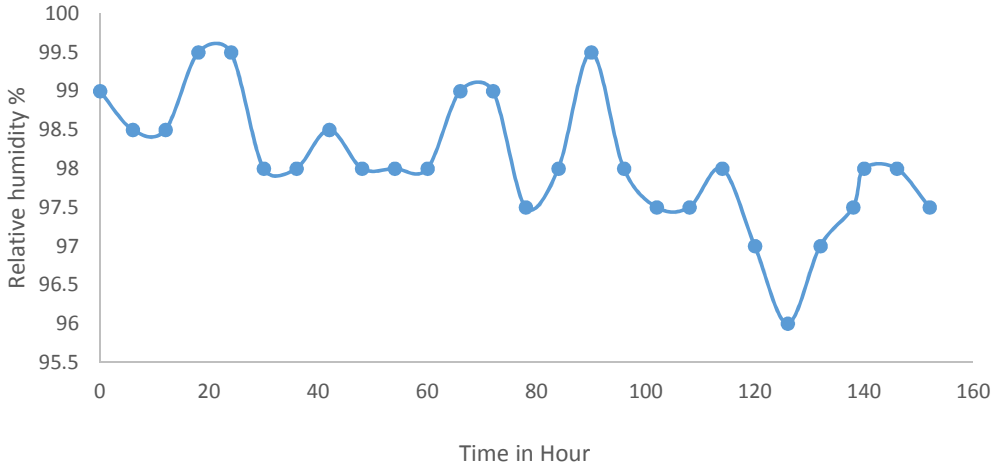

Fig. 10. The relationship between relative humidity and time for stored Bitter leaf

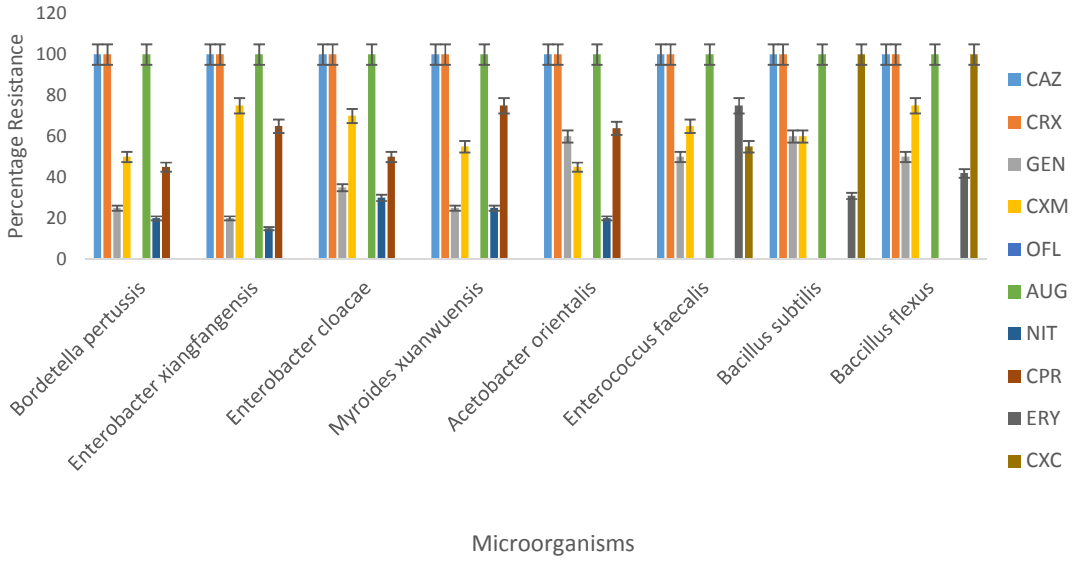

Fig. 11. Percentage resistance of the isolates to different antibiotics

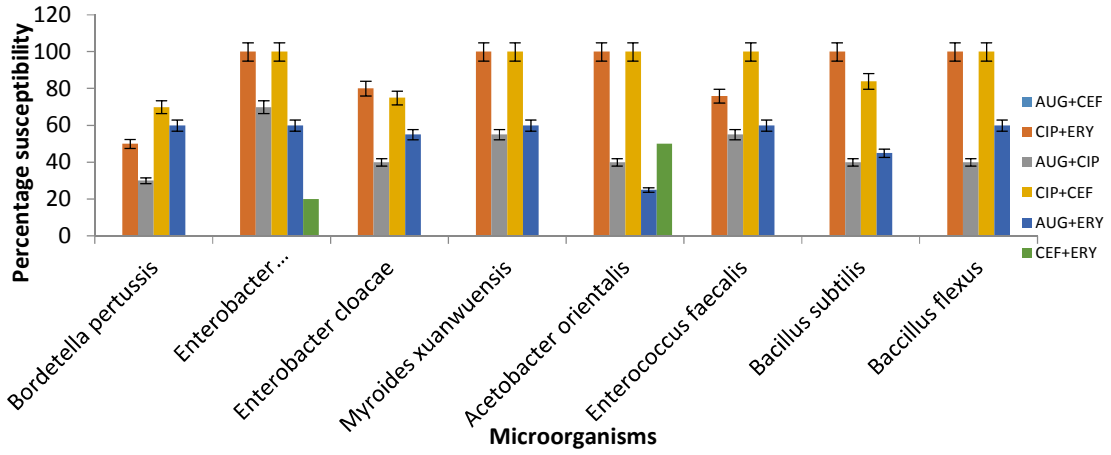

Fig. 12. Combined antibiotic sensitivity of isolates from the vegetables

4. CONCLUSION

Vegetables forms a significant proportion of the diet of many Africans. Apart from its flavouring attribute, it contributes significantly to the intake of minerals, vitamins, fiber, carbohydrates, oils and other micronutrients required for health of persons who eat them. Storage of vegetables is an important factor because of its perishable attributes that has been a challenge especially in the developing countries. The optimum time, temperature, humidity and storage conditions for vegetables can be a limiting factor to predispose the vegetables to rapid deterioration and spoilage. The bacteria identified in this study may be pathogenic especially if the vegetables are consumed raw or not properly cooked. Therefore if t properly developed may have a strong potential in increasing food production, improving the nutritional status of the rural population as well as decreasing food imports.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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