Asian Journal of Immunology

Volume 6, Issue 2, Page 1-10, 2022; Article no.AJI.94762

Antimicrobial Activities of *Citrus aurantiifolia* Peels on Microorganisms Isolated from Spoilt Onions Sold in Awka, Anambra State, Nigeria

Oledibe, Odira Johnson [a*], Ejimofor, Chiamaka Frances [b], Afam-Ezeaku, Chikaodili Eziamaka [a], Nwakoby, Nnamdi Enoch [c], Mbaukwu, Onyinye Ann [a] and Okeke, Somto Francis [a]

[a] Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
[b] Biological Science Department, Chukwuemeka Odumegwu Ojukwu University, ULI, Anambra State, Nigeria.
[c] Microbiology Department, Chukwuemeka Odumegwu Ojukwu University, ULI, Anambra State, Nigeria.

Authors' contributions

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

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/94762

Original Research Article

Received: 16/10/2022
Accepted: 20/12/2022
Published: 22/12/2022

ABSTRACT

One of the most significant monocotyledon crops is the onion, which also contains antioxidants. This research aims to isolate microorganisms that cause spoilage in onion and to study the anti-microbial activities of lime peel extract on the isolates. Spoilt Onion samples were collected from three different markets in Awka Metropolis (Eke Awka, Nnamdi Azikiwe temporary site (Temp. site)

**Corresponding author: E-mail: oj.oledibe@unzik.edu.ng;*

and Amenyi). They were then transported to Alpha research laboratory Awka, for analysis. The spoilt onions samples were sterilized and cultured using the standard procedure. The culture media used for the study were Nutrient Agar and Sabroud Dextrose Agar, both were placed into a conical flask and subjected to an autoclave at a rate of 121ºC at 15psi for 20 minutes and the plates were incubated at room temperature. In the study, various microorganisms that can caused spoilage were identified; the bacteria include K*lebsiella* sp, *E-coli, Staphylococcus spp* and p*seudomonas spp*. The fungal isolates are *Aspergillus sp*, *Penicillium* sp, *Mucor sp, Rhizopus* sp. They were isolated and identified both morphologically and microscopically. Samples from Amenyi had the highest fungal counts ($4.50 \times 10^4 \pm 0.00$a cfu/g) while samples from Eke-Awka had the lowest fungal count ($2.70 \times 10^4 \pm 0.200$c cfu/g) on SDA. Also, samples from the Temp. site had the lowest bacterial counts ($4.71 \times 10^4 \pm 0.100$c cfu/mi) while samples from Eke-Awka had the highest fungal count ($6.10 \times 10^4 \pm 0.03$a cfu/ml). The result showed that ethanol extract from lime peel has good inhibitory effects of the organisms. *Rhizopus* sp. had the highest inhibition at 100% of lime peel extract while *Aspergillus* sp. has no inhibition at 100%, 50%, and 25% of lime peel extract. Also, *Escherichia coli* had the highest inhibition of (43.00 ± 0.30) at 100% of lime peel extract while *Pseudomonas* had no inhibition at 100%, 50%, and 25% of lime peel extract. As a result, the study recommended that farmers utilize lime peel extract, which is a much safer and more effective technique of controlling onion spoilage.

Keywords: Onion; microorganism; lime; media.

1. INTRODUCTION

The family Alliaceae, which also includes leeks, shallots, and garlic, includes the onion (*Allium cepa*). An estimated 88.510 tons of onion bulbs are produced worldwide, with majority produced in Canada [1]. The prevalence of bulb rot disease continues to be a major cause of post-harvest loss in the food and agricultural industries. It has been estimated that in Nova Scotia, the disease bulb rot alone causes the loss of up to 20% of stored onion bulbs [2]. Several pre- and post-harvest factors, including genotypic traits, soil characteristics, climatic factors, and management techniques, can be blamed for the bacterial infection of onion bulbs and the resulting loss of onion bulbs (Gllis et al. 2007). The grey mold fungus, the black mold fungi, the blue mold fungi, and the bacterial rot are onion diseases [3].

Phosphorus, calcium, and carbohydrates are all abundant in onions. The volatile oil known as ally-propyl disulphide is what gives onions their pungent flavor. With a global production of roughly 25 million tonnes, onions are a significant crop on all continents [4].

Bulb rot in onions is brought on by bacterial infections, and it can appear at any point between pre-harvest and storage. The onion's neck softens when pressed, the bulb tissue softens and soaks up water, and the color changes from yellow to brown. The majority of these illnesses or germs affect onions. For the germs to enter and spread, water is necessary. Through wounds and dead lower leaves, bacteria invade the bulb [5,6]. The soil-borne viruses might disperse by irrigation water or rainwater splashes. Warm temperatures (above 85°F) and moist environments are favorable to the majority of these microorganisms that induce onion spoilage [7].

This research intends to isolate and identify the microbes responsible for onion deterioration in several locations in Anambra state, Nigeria. Also, to determine the antimicrobial activities of lime peel extract on these isolates.

2. MATERIALS AND METHODS

2.1 Sample Collection

Three (3) markets in Anambra state (Eke Awka, Nnamdi Azikiwe temporary site (Temp. site), and Amenyi) were where the samples of spoiled onions were purchased. Alpha Research Laboratory in Awka, Anambra state provided the lab and other facilities needed for the practical work.

2.2 Fungal Isolation

2.2.1 Culture media

In this research, Sabouraud Dextrose Agar (SDA), a commercially available media, were used.

2.2.2 SDA media preparation

One liter of distilled water was added to a suspension of around 65g of the medium, which was thoroughly mixed before being heated to boiling and then dissolved. It was heated for one minute to dissolve the solution, and then sterilized for 15 minutes at 118 to 121^0 C in an autoclave. After that, while the solution was still molten, 500 milligrams of the antibiotic streptomycin was added. If any of the solution was left over at that point and wasn't immediately needed, it was kept in the refrigerator between 8 and 15^0 C until then.

2.2.3 Isolation of fungi

To enhance with the dirt removal, distilled water was used to clean spoiled onion surfaces. Using a sterilized scalpel, a little piece of the spoiled fruit was cut off and put onto freshly made PDA and SDA agar, where it was cultured for three (3) days at room temperature.

2.2.4 Sub-culturing techniques

This was achieved using the technique described by Chuku et al. 2017. When more than one colony of fungi was seen in the petri dishes, the resulting colonies were subsequently sub-cultured onto Potato dextrose agar (PDA). This process was continued until pure cultures were obtained.

2.2.5 Identification of isolated fungi

All of the different fungal species that were isolated were identified, and both macroscopic and microscopic aspects as well as their varied properties were examined, including color, texture, hyphae and conidial forms, conidiophore presence, and conidial head shapes. The correct taxonomic keys assisted in the microscopic identification.

2.2.6 Determination of fungal frequency (%)

The prevalence of fungi will be assessed location-wise, in terms of cultural media, and later, in connection to the percentage of diseases calculated based on symptoms. The following formula will be used for fungal frequency percentage determination:

> Fungal Frequency (%) = (Number of particular fungus colonies observed in plates / Total number of colonies of all fungi) x 100

2.3 Isolation of Bacteria

Using nutritional agar and EMB agar, the procedure was utilized to separate the bacteria from the tomato seeds. The seeds (weighing one gram) were aseptically collected before being serially diluted in saline. The agar was inoculated with a mixture of nutrients agar and EMB, and they were incubated at 350ºC for 24 hours in order to count all of the aerobic heterotrophic bacteria and fecal coliforms in the sample.

2.3.1 Total plate count of bacteria (CFU/ml)

The microbial load in agar plate samples was calculated using a formula:

> Cfu/ml = {(No. of colonies X dilution factor) / volume of inoculums)}

2.3.2 Purification of isolates

The selected colonies were then sub-cultured for 24 hours on nutrient agar plates. They were then subjected to microscopic identification and biochemical analyses.

2.3.3 Identification of microorganisms

Morphological identification: The isolated bacteria were identified based on motility and Gram-staining.

2.4 Pathogenicity Test

Pure culture of the fungi was isolated using inoculation loop of length 5cm and sterilized using 100% ethanol. These fungal cultures were subsequently isolated in pure forms by sub-culturing and incubated for 24hrs and used for microscopic characterization and biochemical analysis.

2.5 Statistical Analysis

Analytical Statistics Sigma plot version 12 statistical software was used to conduct a two-way analysis of variance (ANOVA) on the collected data in order to determine the level of significance of the LSD 0.05% treatment.

3. RESULTS

Table 1 showed the colony count of bacteria from the different markets. Eke Awka market had the highest colony count, while the Temp site had the lowest colony count.

Table 2 showed the fungal count from the three markets. Amenyi had the highest fungal count while Eke awka had the lowest.

Table 3 showed that *Escherichia coli* had the highest inhibition of (43.00 ± 0.30) at 100% of lime peel extract while *Pseudomonas* had no inhibition at 100%, 50%, and 25% of lime peel extract.

Table 4 showed that *Rhizopus* sp. had highest inhibition at 100% of lime peel extract while *Aspergillus* sp. had no inhibition at 100%, 50%, and 25% of lime peel extract.

Table 1. Total bacterial count of the Onion Samples

Sample site	Total bacterial count (cfu/ml)
Eke awka market	$6.10 \times 10^4 \pm 0.03$a
Temp site	$4.71 \times 10^4 \pm 0.100$c
Amenyi market	$5.64 \times 10^4 \pm 1.101$b

Table 2. Mean Fungal count of Onion Samples

Sample site	Mean total fungi count (CFU/g)
Eke Awka	$2.70 \times 10^4 \pm 0.200$c
Temp site	$3.15 \times 10^4 \pm 0.100$b
Amenyi market	$4.50 \times 10^4 \pm 0.00$a

Table 3. *In vitro* antimicrobial activity of lime peel extract

Isolates	Lime peel extract 100%	Lime peel extract 50%	Lime peel extract 25%	Std antibiotics 30µg/ml
***Staphylococcus* sp.**	27.00±0.110	14.90±3.01	6.00±0.50	34.83±1.11
Escherichia coli	43.00±0.30	36.00±1.110	30.00±1.00	34.83±0.30
***Klebsiella* sp.**	18.00±1.00	15.70±2.00	11.00±2.00	19.16±1.00
Pseudomonas spp	0.000±0.00	0.000±0.00	0.000±0.00	24.30±0.20

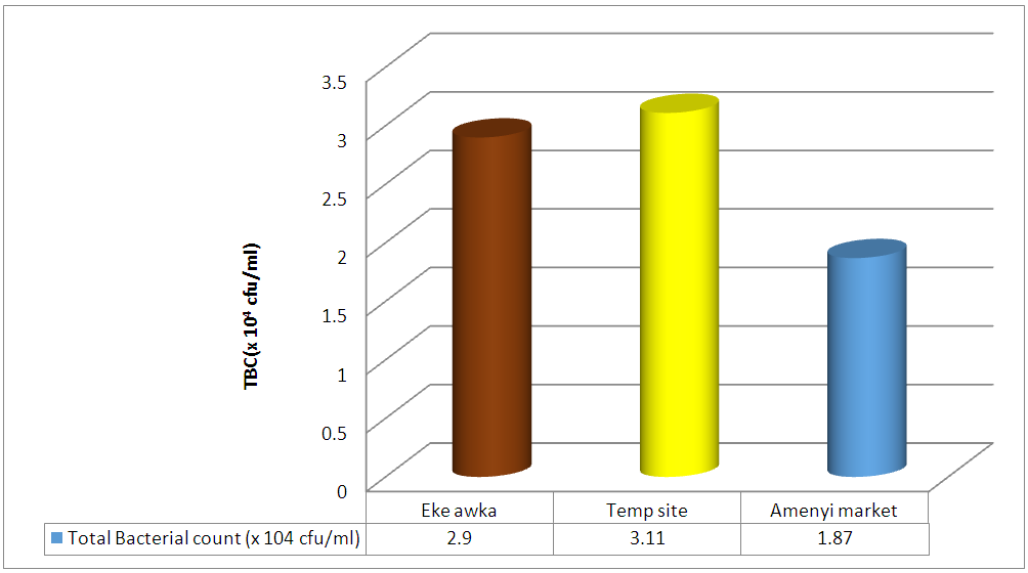


Fig. 1. Total Bacterial count of onion samples

Table 4. *In vitro* antifungal activity of lime peel extract

Isolates	Lime peel extract at 100%	Lime peel extract at 50%	Lime peel extract at 25%	Std antibiotics 30µg/ml
Aspergillus sp.	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00	29.60 ± 1.00
Penicillium sp.	15.00 ± 1.00	12.00 ± 2.00	7.00 ± 1.00	39.00 ± 1.00
Mucor spp	35.00 ± 0.11	23.70.3.01	21.30 ± 3.01	30.00 ± 1.00
Rhizopus sp.	41.00 ± 0.01	29.00 ± 3.01	22.00 ± 0.01	38.00 ± 3.01

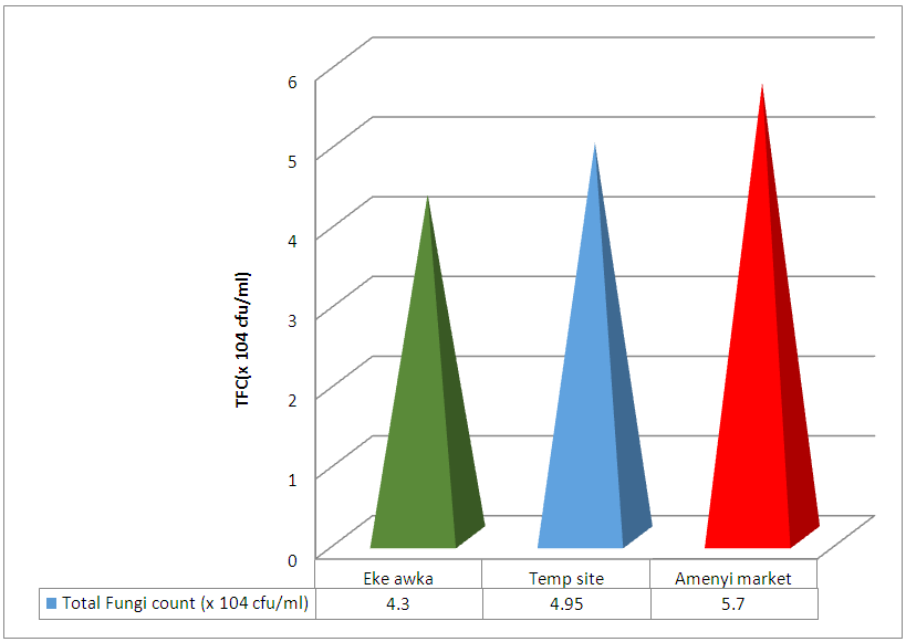


Fig. 2. Total Fungi count of onion samples

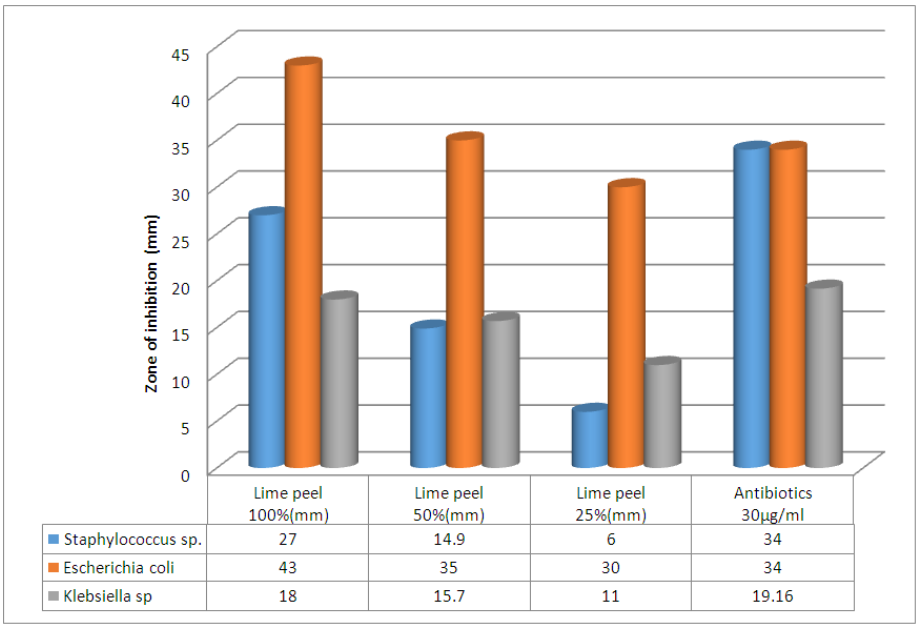
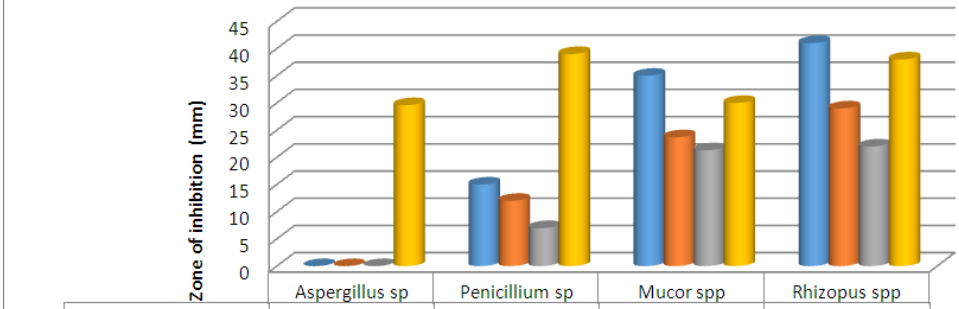


Fig. 3. *In vitro* antibacterial activity of lime peel extract



	Aspergillus sp	Penicillium sp	Mucor spp	Rhizopus spp
Lime peel 100%(mm)	0	15	35	41
Lime peel 50%(mm)	0	12	23.7	29
Lime peel 25%(mm)	0	7	21.3	22
Antibiotics 30µg/ml	29.6	39	30	38

Fig. 4. *In vitro* antifungal activity of lime peel extract

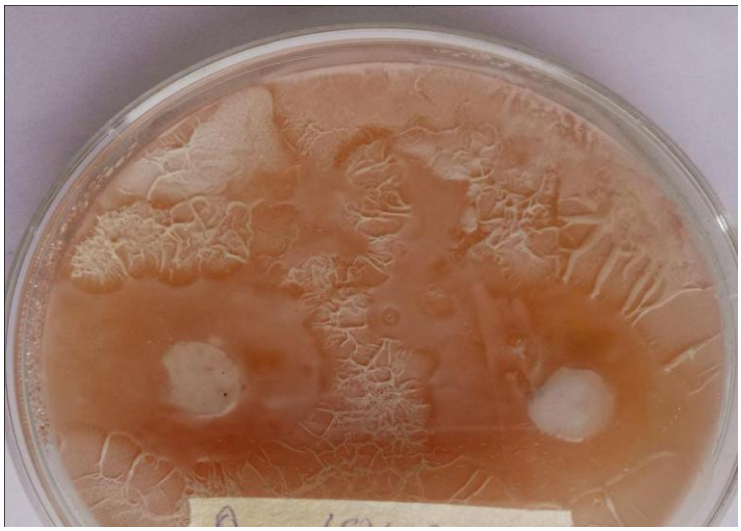


Plate 1. Pure culture of *klebsiella* sp.

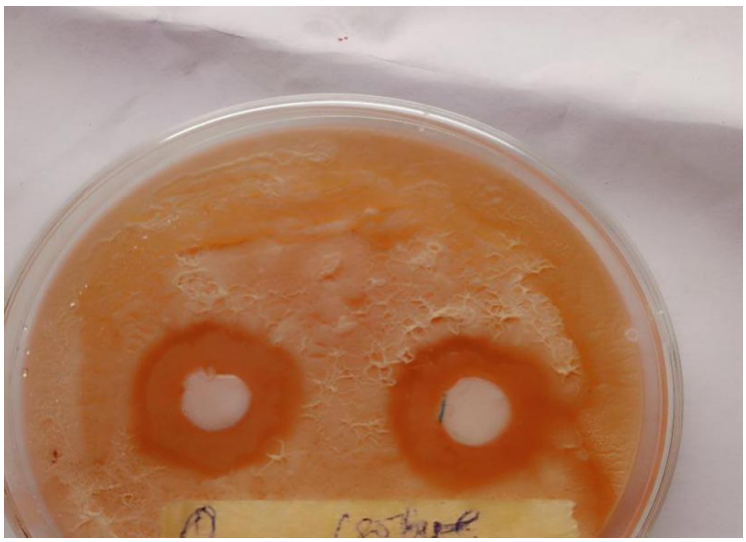


Plate 2. Pure culture of *E- coli.*

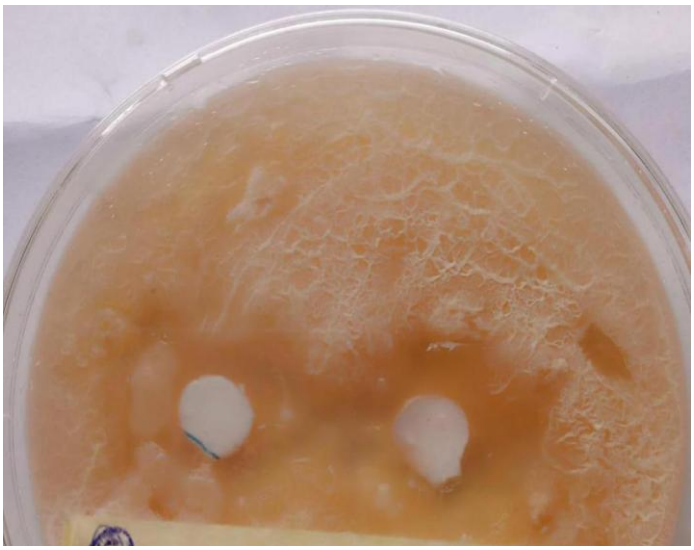


Plate 3. Pure culture of *staphylococcus spp.*

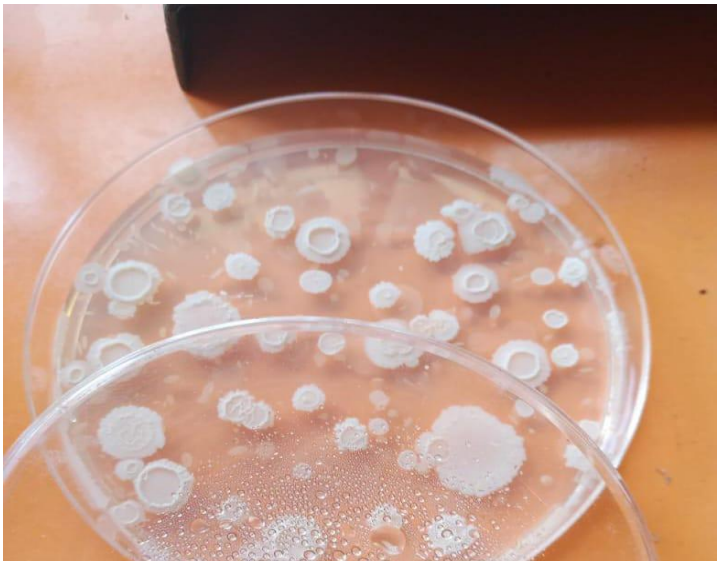


Plate 4. Pure culture of *Penicillium* sp.

4. DISCUSSION

Studies on the microbes that cause onion deterioration in several locations of Anambra State have revealed the existence of a host of microbes. The fungal counts ranged from (2.70, 3.15, and 4.50) $x10^4$ cfu/g) while the bacteria ranged from (4.71, 5.64, and 6.10) $x10^4$ cfu/ml. *Klebsiella* sp, *E- coli, Staphylococcus sp* and *Pseudomonas sp* were the bacteria isolated from the onion samples from the different locations while *Aspergillus sp*, *Penicillium* sp, *Mucor sp, Rhizopus* sp. were the fungal isolates.

The study revealed that there was relatively high incidence of deterioration in the different locations and this can be attributed to pre-harvest handling and storage conditions. This supports the findings of Ghaffor et al. [8] who hypothesized that damage to product caused during harvest and inadequate storage is a major contributor to infection because the majority of spoilage bacteria enter the produce through such damaged tissues. In addition, unsanitary behaviors including cross-contamination and contact infections during product transit might make infections more common [9].

Antioxidants and anti-inflammatory substances are found in onions. Their strong anti-inflammatory capabilities could perhaps lower blood pressure as well [10]. Several pre- and post-harvest factors, including soil characteristics, climatic factors, and management practices, can be blamed for onion bulb infection by microorganisms and the resulting loss of onion bulbs [11].

5. CONCLUSION

Onion (*Allium cepa L.*) is one of the most important vegetable crops commercially grown in the world. Hence, there is a need to develop and promote the use of antiseptics and antibiotics in the control of these microorganisms in the study area.

Although there are many restrictions on growing onions, one of the main issues with growing onions and increasing productivity in the studied area is farmers' inappropriate agronomic methods. Farmers are also encouraged to make use of the liquid substance of lime peel to control microbial growth of onion, either by spraying the onions with lime peel extracts and also take much care during Onion harvest and storage to reduce physical damage which encourage the growth of these bacteria to cause onion spoilage.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Buchanan RE, Gibbons ME. Bergeys Manual of Determinative Bacteriology. 8th ed. Williams and Wilkins, Baltimore. 2014;305-318.
2. Charles PD. *Escherichia coli.* 2012; 0157:H2.
   Available: www.medicinenet.com
3. Barth M, Hankinson TR, Zhuang H, Breidt F. Microbiological spoilage of fruits and vegetables. W. H. sperber, M.P. Doyle (eds.) compendium of the microbiology spoilage of foods and beverages, food microbiology and food safety. *C springer* science + business media, LLC. 2009;35-183.
4. Frazier WC, Westhoff DC. Food microbiology. Mc Graw-Hill publishing Company Ltd., New Delhi. 2015;217-240.
5. Asagbara A, Oyewole OB. Fermentation studies on carrot juice processed to table wine. Nig Food J. 2011;20:74-77.
6. Berger S, Sinha AK, Roitsch T. Plant physiology meets phytopathology: plant primary metabolism and plant-pathogen interactions. J Exper Bot. 2007;58:4019-4026.
7. Dodona SD, Baba KM, Muhammad I, Magaji MD. Evaluation of onion storage losses and implication for food security in Sokoto Metropolis. Bull Sci Assoc Nig, 2005;26:10-14.
8. Ghaffoor A, Jilani MS, Khaliq G, Waseem K. Effect of different NPK levels on the growth and yield of three onion varieties. Asian Journal of Plant Science. 2003;2(3):342–346.
9. Nasir MD, Faridullah KW, Manhaj UD, Shah H.D. Effect of planting density on growth and yield of onion varieties under climatic conditions of Peshawar. Sarhad Journal of Agriculture. 2007;23 (4):912-918.
10. Muhammad A, Gambo BA, Ibrahim ND. Response of onion (*Allium cepa* L.) to irrigation intervals and plant density in Zuru, Northern Guinea Savanna of Nigeria; 2011.
11. Negash A, Mitiku H, Yamoah C. Growth and bulb yield response of onion (*Allium cepa* L.) to nitrogen and phosphorous rates under variable irrigation regimes in Mekelle, Northern Ethiopia. Journal of the Dry Lands. 2009;2(2):110-119.

APPENDIX

Table 1. Morphological and biochemical characteristics of isolates

Parameters	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Colony characterization	Milky circular with flat elevation	Whitish irregular shape with flat elevation	Yellowish irregular shape with flat elevation	Milky irregular shape with flat elevation
Cell characterization	Rod in singles	Rods in clusters	Cocci in clusters	Cocci in clusters
Gram's Test	Positive	Negative	Positive	Negative
Motility Test	Negative	Positive	Positive	Positive
Catalase	Positive	Negative	Positive	Positive
Coagulase	Negative	Negative	Positive	Positive
Citrate	Negative	Positive	Negative	Positive
Indole	Positive	Negative	Negative	Positive
Oxidase	Negative	Positive	Negative	Positive
Urease	Positive	Positive	Negative	Positive
Probable organism	*Klebsiella* sp	*E- coli*	*Staphylococcus spp*	*Pseudomonas spp*

Table 2. List of isolates and their Probable identity

Isolate	Description	Probable identity
Isolate 1	They are Gram-negative bacteria. They are rod-shaped. Identification of *klebsiella* are pink	*Klebsiella spp*
Isolate 2	They are rough or a smooth form. Colonies are rough flat and irregular. *E- coli* are pink in color	*Escherichia coli*
Isloate 3	They are Gram-postive *cocci.* They are non-motile, anaerobic, and catalase-positive, and in pus they form clusters like bunches of grapes	*Staphylococcus spp*
Isolate 4	They are Grams-negative, rod shaped. They are non-sporing bacterium	*Pseudomonas spp*

Table 3. Extraction from different bacteria

Extract	Lime peel extract of 100%	Lime peel extract of 50%	Lime peel extract of 25%	Std antibiotics 30
Staphylococcus sp.	27.00	14.90	6.00	34.83
	27.00	14.50	6.00	34.80
	28.00	14.50	6.00	34.86
Escherichia coli	43.00	35.00	30.00	34.83
	45.00	35.00	28.00	34.00
	40.00	35.00	23.00	34.00
Klebsiella coli	18.00	15.70	11.00	19.16
	18.00	15.80	11.00	20.00
	18.00	15.10	11.00	19.00
Pseudomonas spp	0.00	0.00	0.00	24.30
	0.00	0.00	0.00	24.00
	0.00	0.00	0.00	24.60

Table 4. Total Bacteria count (×10⁴ CFU/ml) from different sites

Sample Site	Total Bacteria count (×10⁴ CFU/ml)
Eke awka	6.10 6.3 6.0
Temp site	4.70 4.74 4.71
Amenyi market	5.60 5.68 5.65

Table 5. Prevalence of Isolates

Collection site	Bacterial isolates
Eke awka	*Klebsiella* sp. *Staphylococcus spp* *Escherichia coli* *Pseudomonas spp*
Temp site	*Staphylococcus spp* *Escherichia coli*
Amenyi market	*Bacillus* sp. *Staphylococcus spp* *Pseudomonas spp*

Table 5 shows that *Staphylococcus spp* has the highest occurrence in the three markets while *Bacillus* sp. has the lowest occurrence.

Table 6. Occurrence of Fungal isolate

Collection site	Fungal isolates
Eke awka	*Aspergillus spp* *Mucor spp* *Rhizopus* sp.
Temp site	*Aspergillus spp* *Mucor spp* *Penicillium*
Amenyi market	*Aspergillus spp* *Mucor spp* *Rhizopus* sp. *Penicillium spp*

Table 6 shows that *Aspergillus spp and Mucor spp* has the highest percentage occurrence while *Rhizopus* sp. and *Penicillium* has the lowest percentage occurrence.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/94762