Afropolitan Journals

ISSN: 2730-7603X www.afropolitanjournals.com

Isolation and Identification of Pathogens Responsible for Post-Harvest Spoilage of Onions (*Allium Cepa L.*) in Some Selected Markets in Maiduguri, Borno State

Mohammed Zanna Malilima and Stephen Peter

Department of Biology, Umar Ibn Ibrahim El-Kanemi College of Education Science and Technology Bama, Borno State.

Corresponding author: mohammedmalilima1968@gmail.com

Abstract

This study aims to identify and isolate pathogens, responsible for post-harvest spoilage of onions (*Allium cepa* L.) with the following stated objectives: To isolate and Identify pathogens responsible for the onion spoilages, to estimate diseased incidence on spoiled onion, to characterizes effect of leaf extract on the pathogens, to determine whether environmental factor has effect on onion post-harvest handling process by farmers. The data was analyzed using simple percentage. physical characteristic and microscopic characteristics of the isolated pathogens; The survey reviles the diseased incidence on spoiled onion isolated from the sampled onions. Five samples sample were collected from three different markets selected for this study. 13.3 percent of the rotten onion are caused by P*enicillium digitatum, Fusarium oxysporum, and Rhizopus stolonifera*. which are mainly fungal pathogens and 26.6 percent is caused by *Aspergills niger* which is also fungi while 26.6 percent *Saccaromyces cerevisiae* which is bacterial isolate. The distribution of the bacteria in relation to the markets surveyed. The percentage distribution was 64.28% *Enterobacter* sp, 21.42% *Pseudomonas* Sp and 14.28% *Escherichia coli,* respectively. *Leaf extract test result of four different disk against standard anti-biotic gentamycin sample.* The neem extract shows total 56 mm inhibition area with averagely 28% against the standard sample gentamycin. Bitter leaf extract also shows 54 total inhibition zone with total 27% while African mahogany with highest inhibition zone shows total 62mm inhibition zone with average of 31%. And Aloe vera shows 60mm total inhibition zone with average of 30%. The research also shows that room temperature (29- 30^0) was the best temperature for development of rot. There was no rot formation at -20^0C and at 4^0c while at 37^0C very little rot developed.

Keywords: Pathogen, Post-harvest, Rotten onion, Bacteria, Fungi, Isolation.

Introduction

Onion (*Allium cepa L.*) belonging to the Family named, Liliaceae. It is universally important culinary vegetable, used on account of its volatile flavor components. It was originally a middle East plant but is nowadays widely cultivated in many parts of America, Europe and Africa for it's sharp-taste, edible bulbs. The leaf bases (Bulbs), which are consumed as vegetables, cooked pickled or eaten raw. They are important for their minerals and vitamin contains and they also add flavor to food. In Nigeria, Onion is grown exclusively in the northern part of Nigeria, are grown during the dry season (October to April). Research shows that there is high intense of onion spoilage, during storages in Northern part of

Nigeria. Often bulbs are attacked by micro-organisms resulting in various spoilage diseases. As a resultant loss from microbial spoilage occur in forms of reduction in the quantity and quality of the produce, as observed by the researcher. The prominent storage diseases of onion bulb have been reported to be caused by fungi such as *Aspergillus niger* (black rot), Botrytis (neck rot) and *Fusarium oxysporum* (bulb-end rot). Brewster JL. (2014) Equally significant are the soft rot diseases of bacterial origin, particularly those that are caused by *Pseudomonas allicola*, *P. marginalia* and *P. cepacea*. Apart from microbial spoilage, the deterioration of raw onions may result from physical factors; actions of their own enzymes, microbial or combination of these factors. Mechanical damage resulting. The present study investigates the microbial species involved in onion bulb spoilage in some selected markets in North-Eastern Nigeria (Borno State).

Problem Statement

The high intense of onion spoilage in northern part of Nigeria attacked by micro-organisms resulting in various spoilage diseases in post-harvest period, which affect farm produce, subsequently cause farmer colossal economic damage. This requires more scientific action in isolation of pathogens responsible for post-harvest spoilage in other to reduce the losses from microbial spoilage occur in forms of reduction in the quantity and quality of the produce. Quantitative pathogenic losses result from rapid and extensive break down of the host tissue by micro-organisms. The pattern at which it attack is usually an initial infection by the host tissues or a few specific pathogens followed by massive infection by a broader spectrum of non-specific 'biodeteriogens which, are only weakly pathogenic or saprophytic on the dead or moribund tissue remaining from primary, infection. Cases reported from some selected onion market in Borno state, discovered that the prominent storage diseases of onion bulb have been reported to be caused by fungi such as Aspergillus niger (black rot), Botrytis (neck rot) and *Fusarium oxysporum* (bulb-end rot). Brewester JL. (2014) Equally significant are the soft rot diseases of bacterial origin, particularly those that are caused by *Pseudomonas allicola, P. marginalia* and *P. cepacea*

Materials and Methodology

Collection of Samples

Onion bulbs showing signs of rotting and discoloration were randomly selected from stock of onion sold at MMC, Bama and Biu Market in Borno State for microbiological analysis. However, average of rotten ones was recorded to estimate the percentage incidence of disease from each of the market sampled.

Isolate and identify the pathogen responsible for onion rots during post-harvest storage;

Aim: To check the presence of pathogenic microbes in food samples (rot onion bulb).

Media: L.S. B SDA, SSA, BPA, PCA. PBA, Peptone water

Apparatus: McCartney bottles, Durham tubes, Autoclave, syringe (10ml and 5ml), incubator and Bunsen burner.

Media preparation: Media were prepared in water according to the manufacturer's instruction.

Peptone water — 1.5g/100ml, LSB — 2.8g/70ml, SSA — 6.04g/75ml, EMB — 2.7g/75ml, BPA — 2.6g/75ml, PCA — 1.7g/ml, SDA — 2.6g/75ml, PDA - 2.8g/75ml

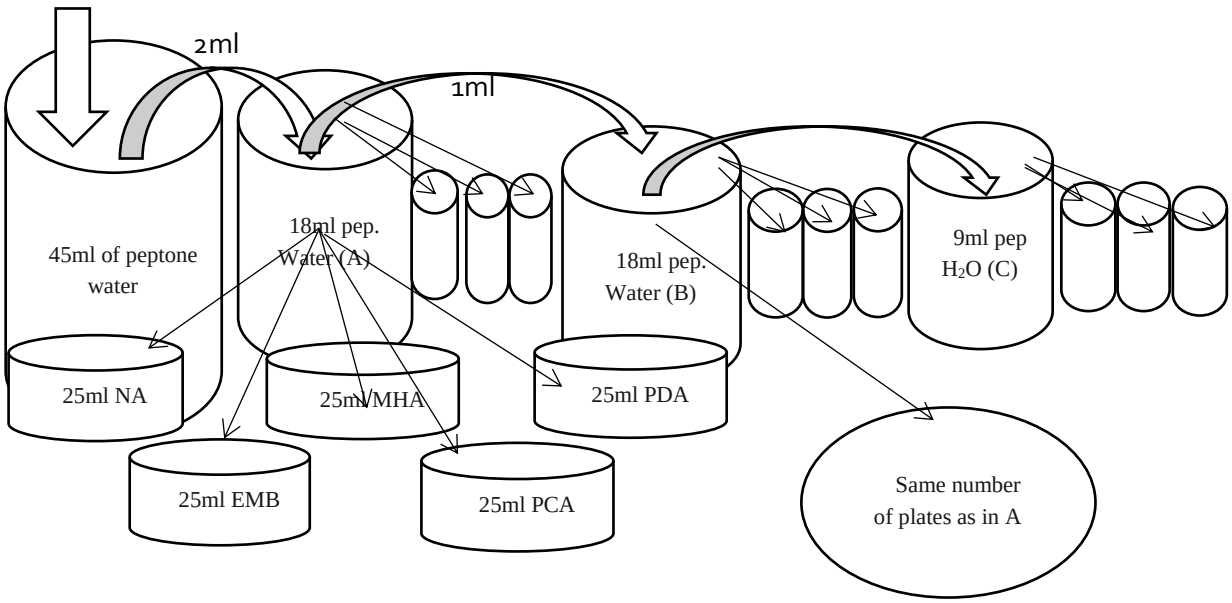
Inoculation

All media were arranged sterilize in autoclave at 121°C (15 MINUTES), and allowed to cool. While SSA was sterilized on burning flame and allowed to cool.

The media plates (Petri-dishes) were labelled A, B and control for each from solution A, 1ml of the samples was transferred respectively to correspondent plate to A. and from B 1ml was transferred to all plates marked B. After these, 25ml of the media was added to the plates that have corresponding labelling and were allowed to gel.

Similarly, 1ml each of solution A was transferred to 3-bottles labelled A, the same thing was done for solutions B and C and they were capped. The Durham tubes in all the bottles were filled, inverted and arranged into the incubator. This parameter was repeated for each sample. 1,2,3,4,5 from MMC market, biu and bama respectively.

10g of sample



Source: National Agency for Food and Drug Administration and Control NAFDAK Kaduna area Lab. S.O.P 2017.

The plates were incubated at room temperature (25- 28°C) for 5 days for growth. To isolate bacterial pathogens from the rotting onion bulb, the Plate Count Agar was used. Sample

was picked from the rotting part of the onion bulb and inoculated onto the solid medium by streak method. Plates were incubated at 37°C for 24 hours. *Obiageli et al.; MRJI, 29(4): 1-6, 2019; Article no. MRJI.501633*

Diseased Incidence on Spoiled Onion

Percentage diseases incidence was calculated using the formula bellow;

Diseases incidence = percent $= \frac{\text{Numbers of Infcted Bulbs}}{\text{Toatl Numbers of Bulb Collected}} \times 100$

Characterizes effect of leaf extract on the organisms

Neem leaf extract, bitter leaf extract, mahogany leaf extra and aloevera leaf extract was used to determine the susceptibility of various bacterial and fungi species to various antibiotics and synthetic agents. A standardized disk diffusion method was used; antibiotic standard sample susceptibility testing and the zone size interpretation chart were used for the determination of the bacterial sensitive to the antibiotics selected.

Effect of Environmental Factor on Onion Post-Harvest Handling Process by Farmers

Effect of temperature: Five healthy onions bulbs were peeled and surface sterilized with 70% (V/V) ethanol. Five wounds were made on each bulb using a sterile cork-borer on the first leaf based on the wound were inoculated 0.05ml aliquots containing 3.2 x 10^9 number of cells. Control bulbs were inoculated with same amount of sterile distilled water. After inoculation, the bulbs were placed in sterile glass jars and placed in incubators kept atn4°C, 29 +2° 37°c and 44°c for one month. Development and extent of rot were observed

Effect of relative humidity: Six healthy onion bulbs were peeled and surface. Sterilized with 70% (v/v) ethanol. A wound was made on the outermost fleshy leaf base of each bulb with a cork borer. Into this wound was inoculated 0.05ml aliquot containing 3.2 x 10. The control bulbs were inoculated with same amount of sterile distilled water. Each bulb was placed on a glass ring inside sterile glass jar and placed in a relative humidity chamber. In the lower part of the chamber was placed 100ml of the appropriate saturated salt solution corresponding to 95, 90, 85, 80, 75, 70, 65, 60, 55 and 50% relative humidity at 29°c (Wins ton and Bates, 1960).

The extent of rot at various relative humidifies was determined by measuring the radius of rot from point of inoculation.

Analysis and Design

Isolated and Identify pathogens responsible for the onion spoilages

Table 2: Isolated fugues pathogens in three different onion markets in Borno state

Organism	MMC	Bama	Biu
Aspergillus fusarium oxysporium	0	1	1

Sacharomyces cerevisiae	1	0	0
Penicillium digitatum	**0**	**0**	**1**
Rhizopus tolonifer	0	1	1
Aspergillus niger	1	2	1
%	28.57	42.85	28.57

Table 3. The physical and microscopic characteristics of fungi isolated from onion bulbs

Physical characteristics	Microscopic characteristics	Fungi isolated
Greenish-grey colony	septate aerial conidiophore that is perpendicular	*Penicillium digitatum*
Pink in center with white edge	Mucor conidia, ovoid to ellipsoidal, slightly curled and pointed at both ends	*Fusarum oxysporium*
Grey, smooth, moist colony	Septate mycelia with branching Sporongiospores	*Saccharomyces cerevisiae*
Pale-white colony later turned brown-black	Non-septate mycelia with ovoid Shape	*Rhizopus stolonifera*
Pink-like black wide colony with white edges	Large conidia heads, dark brown becoming radiat and split to columns	*Aspergillus niger*

Source: Obiageli et al.; MRJI, 29(4): 1-6, 2019; Article no. MRJI.50163

The table above shows the characteristic observed in isolated fungus*: Penicillium digitatum*; Greenish-grey colony, septate aerial conidiophore that is perpendicular, *Fusarum oxysporium:* Pink in center with white edge, Mucor conidia, ovoid to ellipsoidal, slightly curled and pointed at both ends. *Saccharomyces cerevisiae:* Grey, smooth, moist colony, Septate mycelia with branching Sporongiospores. *Rhizopus stolonifera:* Pale-white colony later turned brown-black, Non-septate mycelia with ovoid Shape. *Aspergillus niger:* Pink-like black wide colony with white edges, Large conidia heads, dark brown becoming radiat and split to columns

Table 4: Morphology of Isolated bacteria pathogens in different onion selected

Isolation	Form	Size	Margin	Elevation	Color of colony
Escherichia coli	circular	large	undulate	flat	*Metallic- green*
Enterobacter Spp	*Round*	Small	Entire	Slightly raised	*Yellowish-milky*
Pseudomonas Spp	*Circular*	Small	Entire	Raised	*Yellowish –white*

Source: Obiageli et al.; MRJI, 29(4): 1-6, 2019; Article no. MRJI.50163

Table 5. Grams and Biochemical reactions of bacterial isolates Cell Morphology Grams Reaction Catalase Citrate Coagulase Indole Motility Possible organism

Rods	+	+	-	-	+	+	*Escherichia coli*
Cocci	-	-	-	-	+	-	*Enterobacter sp*
Rods	-	+	+	-	-	-	*Pseudomonas*

Source: Obiageli et al.; MRJI, 29(4): 1-6, 2019; Article no. MRJI.50163

Diseased Incidence on Spoiled Onion

Diseases incidence = percent = $\frac{\text{Numbers of Infcted Bulbs}}{\text{Toatl Numbers of Bulb Collected}}$ x 100

Table 6. Percentage distribution of the fungi from the spoilt onion bulbs

Fungi	Number of isolates	% distribution
Penicillium digitatum	2	13.3
Fusarium oxysporum	*2*	*13.3*
Rhizopus stolonifer	*2*	*13..3*
Aspergillus niger	*4*	*26.6*
Saccharomyces cerevisiae	*1*	*6.6*

The table 6. Above shows that 13.3 percent of the rotten onion are caused by P*enicillium digitatum, Fusarium oxysporum, and Rhizopus stolonifera.* which are mainly fungal pathogens and 26.6 percent is caused by Aspergills niger which is also fungi while 26.6 percent *Saccaromyces cerevisiae* which is bacterial isolate.

Table 7. Percentage distribution of the bacteria in the markets

Market	*Enterobacter* sp(n)	*Pseudomonas* sp (n)	*Escherichia coli* (n)	% distribution
MMC	1	1	0	13.3
Biu	2	1	1	26.6
Bama	2	1	1	26.6

The distribution of the bacteria in relation to the markets is presented in Table 7. The percentage distribution was 13.3%, from MMC market, 26.6% from Biu market and Bama respectively.

Table 8. Percentage distribution of bacteria isolates from the spoilt onion bulbs

Bacteria	Number of isolates	% distribution
Enterobacter sp	*5*	*64.28*
Pseudomonas sp	*3*	*21.42*
Escherichia coli	*2*	*14.28*

The percentage distribution of the bacteria in the spoilt onion bulbs is shown in Table 8. The percentage distribution was 64.28% *Enterobacter* sp, 21.42% *Pseudomonas* sp and 14.28% *Escherichia coli,* respectively.

Characterizes effect of leaf extract on the organisms

Table 9: characteristics of leaf extract on the isolated pathogen Aspergillus *niger*, *Escherichia coli*, *Pseudomonas* sp

Plates	Neem leaf extract	Bitter leaf Extract	African mahogany leaf extract	Aloevera Leaf extract	Standard gentamycin anti-biotic
1) A. niger	28mm	27mm	31mm	30mm	35mm
2) E.coli	28mm	27mm	31mm	30mm	35mm
TOTAL	56	54	62	60	70
AVERAGE	28	27	31	30	

Table:9. Summary of potency test result of four different disk against standard anti-biotic gentamycin sample. The neem extract shows total 56 mm inhibition area with averagely 28% against the standard sample gentamycin. Bitter leaf extract also shows 54 total inhibition zone with total 27% while African mahogany with highest inhibition zone shows total 62mm inhibition zone with average of 31. And aloeveera shows 60mm total inhibition zone with average of 30%.

Effect of Temperature

Table 10: The Effect of temperature on onion rot development by P. Fluorescens after 7-day s incubation

Temperature (°c)	Diameter of rot (cm)
Control	0
4	0
44	0
-20	0
29 ± 2	4,36 ± 0.17
37	0.21 ± 0.14

Plate 14: onion bulb inoculated with P. fluorescence and distilled water they were incubated at 44° C for 7days. However, the table 10. Above shows that room temperature (29- 2°0) was the best temperature for development of rot. There was no rot formation at -20°C and at 4°c while at 37°C very little rot developed.

Effect of Relative Humidity

High relative humidity favored development of rot. Rot development was not observed at 50%. R.H. Fifty percent rot was observed at 85 -90% R.H. (table 7).

Table 11: Effect of relative humidity on onion rot development after incubation for 2days

R.H.%	Diameter of rot (CM)	% maximum rot
Control	0	0
50	0 ± 0.019	0
55	0.06 ± 0.04	22.3
60	0.15 ± 0.09	22.8
65	0.27 ± 0.10	23.4
70	0.42 ± 0.13	25.7
75	0.68 ± 0.21	22.9
80	1.38 ± 0.22	31.7
85	2.38 ± 0.21	46.5
90	2.99 ± 0.15	46.9
95	3.40 ± 0.16	88.4
100	4.35 ± 0.20	100

The greatest amount of rot was assigned 100% and the lesser amount of rot expressed as percentage of it.

Summary and Recommendation

Summary

From the analysis table 1; show the pathogens isolated from the collected samples of onion in MMC, Biu and Bama market in Brno state, which are *Aspergillus fusarium oxysporium (0) in MMC, (1)* in Biu, *(1)* In Bama. *Sacharomyces cerevisiae (1)* in, (0) in Bama, (0) in Biu, *Penicillium digitatum(0) in MMC, (0) in Bama, (1) in Biu Rhizopus tolonifer: I(0) in MMC, (1)in Bama, (1) in Biu, Aspergillus niger:(1) in MMC, (2) in Bama, (1) in Biu*, ***Enterobacter* sp**:(1) in MMC, (2) in Bama, (2) in Biu, *Pseudomonas* sp (1) in MMC, (1) in Bam, (1) in Biu, *Pseudomonas Spp.* this is in-line with the studies of ; Mohamed H. Abd-Alla, Shymaa R. Bashandy Stefan Ratering & Sylvia Schnell, Shehu K, Muhammad S. and Sang Hye Ji, Tae Kwang Kim, Young Soo Keum & Se-Chul Chun.

Table 3 and 4. Above shows the physical characteristic and microscopic characteristics of the isolated pathogens; from the table 1 shows that *the characteristic observed in isolated fungus Penicillium digitatum* ; Greenish-grey colony, septate aerial conidiophore that is perpendicular, *Fusarum oxysporium:* Pink in center with white edge, Mucor conidia, ovoid to ellipsoidal, slightly curled and pointed at both ends. *Saccharomyces cerevisiae:* Grey, smooth, moist colony, Septate mycelia with branching Sporangia-spores. *Rhizopus stolonifera:* Pale-white colony later turned brown-black, Non-septate mycelia with ovoid Shape. *Aspergillus niger:* Pink-like black wide colony with white edges, Large conidia heads, dark brown becoming radiate and split to columns. This is also in-line with the investigations of Ozoude, T. Obiageli, Igbokoyi, R. Oluwaseun1, Okey-Ndeche N. Florence1 and Eleanya, E. Ukachi*:* Table 5. Shows the staining characteristics of isolated bacteria. Table 5, 6 and 7 shows the diseased incidence on spoiled onion isolated from the sampled

onions. The table 6. Above shows that 13.3 percent of the rotten onion are caused by P*enicillium digitatum, Fusarium oxysporum, and Rhizopus stolonifera.* which are mainly fungal pathogens and 26.6 percent is caused by *Aspergills niger* which is also fungi while 26.6 percent *Saccaromyces cerevisiae* which is bacterial isolate. The distribution of the bacteria in relation to the markets is presented in Table 7. The percentage distribution was 13.3%, from MMC market, 26.6% from Biu market and Bama respectively, the percentage distribution of the bacteria in the spoilt onion bulbs is shown in Table 8. The percentage distribution was 64.28% *Enterobacter* sp, 21.42% *Pseudomonas* sp and 14.28% *Escherichia coli,* respectively. *Table:9*. Summary of potency test result of four different disk against standard anti-biotic gentamycin sample. The neem extract shows total 56 mm inhibition area with averagely 28% against the standard sample gentamycin. Bitter leaf extract also shows 54 total inhibition zone with total 27% while African mahogany with highest inhibition zone shows total 62mm inhibition zone with average of 31. And aloe-vera shows 60mm total inhibition zone with average of 30%. the research also shows that shows that room temperature (29- 2°0) was the best temperature for development of rot. There was no rot formation at -20°C and6 at 4°c while at 37°C very little rot developed from the table 10.

Recommendations

- There should be more research into locally available means of controlling post-harvest pathogenic spoilage of onion to save the economic lost by the farmers
- More knowledge of post-harvest should be advertised to farmers, indoor to save them from the economics loss of the produces

Acknowledgement

This research is finally completed in honor of the entire members of Umar Ibn. Ibrahim El-Kanemi College of Education Science and Technology UIICEST Bama, Borno State, Nigeria, and the learning peers that will find this research useful in the course of their leaning.

References

Brewester JL. *Onions and Other Vegetable Alliums*: 2nd Edition crop production science in agriculture CABI; 2014.

Dimka SO, Onuegbu BA. *Mycoflora of copra and effect of brining on some properties of copra in Nigeria.* Agricultural and Biology Journal of North America. 2012;6(2):595-352.

Mohamed H. Abd-Alla, Shymaa R. Bashandy Stefan Ratering & Sylvia Schnell; *First Report of Soft Rot of Onion Bulbs in Storage Caused by Pseudomonas Aeruginosa in Egypt:* Journal of Plant Interactions ISSN: 1742-9145 (Print) 1742-9153

Roopa VM, Suvarna VC. *Bio-preservation of onions using plant extracts and antagonistic organisms*. 2014;4(6):962-418.

Sang Hye Ji, Tae Kwang Kim, Young Soo Keum & Se-Chul Chun: *The Major Postharvest Disease of Onion and Its Control with Thymol Fumigation During Low-Temperature Storage*: Mycobiology ISSN: 1229-8093 (Print) 2092-9323

Shehu K, Muhammad S. *Fungi associated with storage rots of onion bulbs in Sokoto, Nigeria*. International Journal of Modern Botany. 2013;1(13):2013-5923.

Yurgel SN, Abbey L, Loomer N, Gillis-Madden R, Mammoliti M. *Microbial communities associated with storage onion*. Phytobiomes. 2018;*2*(1):35-41.

Ozoude, T. Obiageli, Igbokoyi, R. Oluwaseun1, Okey-Ndeche N. Florence1 and Eleanya, E. Ukachi*: Evaluation of Pathogenic Microorganisms that Cause Onion Bulb Rots in Selected Markets in Abuja, Nigeria; M*icrobiology Research Journal International 29(4): 1-6, 2019; Article no. MRJI.50163 ISSN: 2456-7043