Isolation and identification of fungi in onion bulbs obtained from markets in Awka metropolis, Anambra state, Nigeria

Ikeh, M.I*[1]., Ishar, C.O[1]., Eriobu, C.C[1]., Okeke, O.A[1]., Nnatuanya, I.O[1]

[1*]Public Health and Environmental Research Group (PUHEREG), Department of Zoology, Faculty of Biosciences, Nnamdi Azikiwe University Awka

Submitted: 10th April, 2023; Accepted: 23rd April, 2023; Published: 30th April, 2023
DOI: https://doi.org/10.54117/jcbr.v3i2.12
**Corresponding author:Ikeh, M.I; drifeanyiikeh2@yahoo.com; +2348037457581*

Abstract

Onion is one of the most important spice crops cultivated for its nutritious content for consumption and economic value globally. The study was carried out to investigate the fungi microbes associated with spoilage of onion bulbs around Awka metropolis. Onion bulbs with visible signs of spoilage were collected from four different markets in Awka namely; Eke Awka, Amaenyi, first and second markets Ifite and were cultured for the presence of fungi. A total of twenty (20) onion samples, five (5) from each study site were used. Samples were examined using various culture media, and Germ tube test. Data was analyzed using one-way ANOVA and chi-square test. The fungal organisms isolated were *Saccharomyces spp, Fusarium spp, Rhizopus spp, Mucor spp, Aspergillus niger* and *Candida tropicalis*. The fungus, *Aspergillus niger* had the highest percentage distribution of 30% while *Candida tropicalis* and *Mucor spp* had the least percentage of 10% each. The mean total colony count for fungi was highest in second market Ifite (66.80 ± 5.16) but lowest in Amaenyi market (45.60 ± 6.73). Some of the isolated fungi in this study have serious public health importance while others fasten spoilage of the onion bulbs. It is recommended that both farmers, marketers and consumers should take precautionary measures in preventing contamination and consumption of fungi contaminated onion.

Key words: Isolation, Onion bulbs, Fungi, Culture media, Awka

Introduction

Onion (*Allium cepa L*) is an essential vegetable crop in Nigeria based on its nutritional and economic value. It is one of the most important and familiar spice crops grown throughout the globe (Onuorah and Obika, 2015). The crop is grown for its bulbs which are harvested in most countries once a year and are used daily in every home for seasoning and flavouring of foods. Onion is a valuable ingredient in the diet due to its content of sugars, vitamins, minerals, electrolytes, proteins and dietary fibre (Velez *et al*., 2004; Benkeblia, 2004; Ole *et al*., 2004). These include various phytomolecules such as flavonoids, sulphur compounds, phenolic acids, ascorbic acid and polyphenolic substances that are responsible for its aroma, flavor and color (Dorrigiv *et al*., 2021). It is cultivated for its mild flavours and forms an essential ingredient in the diet of many people. The distinctive character of onion is due to the presence of alliaceous odour which accounts for its use as food, salad, spices, condiment and in medicine

(Raju and Naik, 2007). Numerous benefits have been attributed to onion including prevention of cancer and cardiovascular disorders, reduction in the blood levels of cholesterol, reduction in osteoporosis (Adebayo and Diyaolu, 2003), reduction in stomach ulcers, inhibition of the proliferation of cultured ovarian breast and colon cancer cells, inhibition of platelets mediated thrombosis, prevention of inflammatory processes associated with asthma, treatment of fever, common cold, cough, sore throat and its uses as antimicrobial agent (Tyson and Fullerton, 2004). Studies have shown that onion extract is very effective in cardiovascular disease because of their hypocholesterolemic, hypolipidemic, anti-hypertensive, anti-diabetic, anti-thrombotic and anti-hyperhomocysteinemia effects, and also possess many other biological activities including antimicrobial, antioxidant, anti-carcinogenic, anti-mutagenic, anti-asthmatic, immunomodulatory and prebiotic activities (Corzo-Martinez *et al*., 2007).

Onions are locally packed in baskets and jute bags. These packaging materials come from pal, bamboo and fibrous jute trees. However, these materials provide no barriers against dusts and can easily be crushed which might lead to damage of the onions. During storage, losses occur due to sprouting, drying and rotting (Muhammed *et al*., 2004). Bulb rots are a common cause of onion loss during storage. They are caused by microorganisms particularly fungi. For instance, the black mould disease caused by *A. niger* is a limiting factor in onion production globally (Raju and Naik, 2006). *A. niger* has been reported to survive between onion crops as a soil saprophyte or in bulbs in field or storage. The fungus invades bulbs of onions in field or storage whenever they find injured tissues by producing various enzymes or toxins (Raju and Naik, 2006). The association of *A. niger* and other fungi organisms cause 30-80 % loss or spoilage of onion bulbs (NPCC, 2006). Many studies have documented spoilage of onion bulbs induced by microorganisms in different parts of the country and worldwide. Although similar studies have been conducted in some other parts of Awka by other researchers such as Onuora and Obika (2015), it has become necessary to update available literatures due to the increasing effect climate change on fungal activities especially in the tropics (Nnadi and Carter, 2021). For instance, our study which was conducted in Awka like the previous study has revealed the presence of new fungal species involved in onion spoilage which were not found by the previous study.

Materials and Method

Study Area

The study was conducted in Awka south local government area of Anambra state, Nigeria. Awka which is the capital of Anambra state is located between latitudes 06° 06’ N and 06° 16’ N and longitudes 07° 01’ E and 07° 10’ E. The area lies within the tropical rainforest zone of West Africa with an average humidity of 80 %. The city has a mean daily temperature of 20°C and a mean annual rainfall of 200 cm (Emengini *et al*., 2014).

Sample Collection

The onion bulbs with visible signs of spoilage were randomly obtained from four different markets in Awka namely; Amaenyi market, Eke-Awka market, first and second markets Ifite respectively. The onion bulbs were packaged in different sterile polythene bags and transported to the laboratory for analysis.

Material Sterilization

Different laboratory wares such as petri dishes, test tubes, conical flasks, beakers and universal bottles were used for this study. The wares were washed with detergent, rinsed with clean water and air dried. This was followed by sterilization of the apparatus in a hot air oven at high temperature of about 160°C for 60 minutes. The entire working surfaces were also disinfected with ethanol to reduce contaminants.

Media Preparation

The media used for the isolation and characterization were Sabouraud Dextrose Agar (SDA) (Himedia Laboratories Pvt Ltd, India) , Nutrient agar (Chaitanya Agro Biotech Pvt Ltd, India), MacKonkey agar (Titan Biotech Ltd, India), Nutrient broth (TM Media, India), Eosin methycylene blue, Peptone water (Titan Biotech Ltd, India), Urea agar (HKM, China), Plate count, Citrate agar (Himedia, India), Agar agar (Ingreland, China), Triple sugar iron agar (Huankai Microbial, China), Methyl red (Suvchem, India) and Voges proker (Voges Gemuse GmbH, Germany) respectively and were prepared according to instructions.

Serial Dilution and Sample Preparation

Approximately 70% alcohol was used to wipe the surface of the onion bulbs. About 1g of the onion bulb was cut using a sterile razor blade from each sample and was transferred separately into a sterile mortar and pestle, sterile distilled water was added into the mortar and crushed. One millilitre (1 ml) of the sample was added into 9 ml of the sterile nutrient broth in universal bottles for each sample and was allowed to stay for 15minutes. One millilitre (1 ml) from each of the test tube was used to carry out serial dilution for each sample

Fungi Isolation

The spread plate technique was employed. Aliquots (1ml) of the serially diluted sample (10^3) was spread on the surface of sabouraud dextrose agar (SDA) contained in a sterile petri dish. The petri dish also had two percent chloramphenicol added to inhibit bacterial growth. Incubation was carried out in an inverted position at 28°C for five days. The fungal colonies that developed were purified by repeated subculturing on sterile SDA and later stored on SDA slants for characterization and identification (Onuorah and Obika, 2015).

Microbial Colony Count

Fungal count was carried out on each of the plate to determine the number of fungal growth. This was obtained by counting the whole plate and using the number obtained to multiply by the dilution factor (Orpin *et al*., 2017).

Macroscopy and Colonial Identification

Characterization and identification of the colony isolates was achieved by initial morphological examination of the colonies in the plate (macroscopy) for colonial appearance, size, elevation, form, edge, consistency, colour, opacity, haemolysis and pigmentation and results recorded accordingly. The isolates were identified and characterized based on their cultural characteristics, gram and germ tube reaction.

Identification of Fungi (Gram Reaction)

This was carried out to differentiate gram positive from gram negative organisms. A little portion of the fungal growth was placed on a clean glass slide, a drop of the Lactophenol Cotton Blue (LPCB) was added , the speck was emulsified on the slide and viewed using 10x and 40x objective lens (Cheesbrough, 2006).

Germ Tube Test

The test was performed as described by Menza *et al*. (2013). A colony of the test yeast cell was inoculated in human serum and incubated at 37°C for three hours. A drop of the incubated serum was placed on a microscope slide and covered with a coverslip and examined under the microscope for the presence of germ tube.

Statistical Analysis

All experiments were carried out in duplicates. Frequency of occurrence of fungi isolates were tabulated as simple percentages. Data was analyzed using one way analysis of variances (ANOVA) and means were compared using the least significant difference (LSD).

Results

From the 20 onion bulbs examined, the highest fungal colony count was observed in second market with a mean of 66.80 and standard error of ±5.16 while the lowest fungal colony count was observed in Amaenyi market with a mean of 45.60 and standard error of ±6.73. However, there was no significant difference (P>0.05) between the means of the different markets (Table 1).

Table 1: Mean total viable count of fungi in onions in the different markets

Markets	Mean total viable count
Eke-Awka	50.00 ± 3.70^{a}
Amaenyi	45.60 ± 6.73^{a}
First market Ifite	48.20 ± 7.71^{a}
Second market Ifite	66.80 ± 5.16^{a}

∗Mean ± standard error of means

∗Means in columns with similar superscripts are not significantly different from each other (P>0.05)

Based on the number of occurrence of organisms isolated, the fungus, *Aspergillus niger* (6) and *Mucor spp* with *Candida tropicalis* (2) had the highest and lowest occurrences respectively (Table 2).

Table 2: Number of occurrences of the fungal organisms isolated

Fungal organisms	Number of Occurrence	Percentage (%)

Aspergillus niger	6	30
Fusarium spp	4	20
Rhizopus spp	3	15
Mucor spp	2	10
Candida tropicalis	2	10
Sacchromyces spp	3	15
Total	20	100

Various fungi organisms identified and characterized from the spoilt onion samples included *Aspergillus niger, Rhizopus spp, Fusarium spp, Mucor spp, Candida tropicalis* and *Sacchromyces spp. Candida tropicalis, sacchromyces spp* and *Mucor spp* appeared whitish on Sabouraud Dextrose Agar plate and thick non-septate hyphae on microscope. The two (2) yeast species (*C. tropicalis* and *Sacchromyces spp*) appeared as small, creamy or whitish colonies that were somewhat more raised than bacteria colonies. *A. niger* appeared as black colonies with whitish edges. *Rhizopus spp* appeared as whitish colonies with black dots while *Fusarium spp* appeared as yellowish colonies (Table 3).

Table 3: Identification and characterization of fungi isolated

Fungal organisms	Microscopic observation	Morphological characteristics
Aspergillus niger	Presence of septate hyphae and long unbranched sporangiospores with large rounded head	Creamy to brownish-black mycelium with dark spores. Black colonies with white edges
Rhizopus spp	Visible spores and microconidia with septate hyphae	White cottony mycelia with black dots that covers the entire plate
Fusarium spp	Presence of dark pigment of microconidiophores which is spherical in shape	Presence of sickle shaped microconidia that is white to yellow in colour
Mucor spp	Visible spores and sporangiospore with non-septate hyphae	White heavy, woolyfluffy growth covering the entire plate
Candida tropicalis	Single clusters of blastoconidia which is round and enlongated	Creamy to yellowish colonies with smooth surface

Saccharomyces spp	Oval in shape with very short multilateral budding	Small creamy or whitish colonies

Discussion

Studies on the microorganisms associated with the spoilage of onion bulbs obtained from different markets in Awka revealed quite a number of fungi organisms. Onion bulbs, being perishable, contain about 86.8% of moisture and form an ideal medium for proliferation of many storage fungi (Srinivasen *et al*., 2002). In the present study, the fungal organisms isolated were *Mucor spp, Rhizopus spp, Fusarium spp, Aspergillus niger* and two yeast species namely *Candida tropicalis* and *Sacchromyces spp*. Similar findings were also reported by Adongo *et al*., (2015) in Kumasi metropolis Ghana, Shehu and Muhammad (2011) in Sokoto State Nigeria, Ara *et al*., (2008) in Bangladesh, Rajapakse and Edirimanna (2002) in Sri Lanka, with the exception of the presence of *C. tropicalis* and *S. cerevisiae*. However, they have reported the presence of *A. flavus, A. fumigatus, Alternaria porri, Colletotrichum spp* and *Penicillium spp* in the rotten tissues of onion bulbs they examined in addition to the fungal species isolated in this study. This may have been as a result of the different geographical locations, temperature, humidity and other weather condition and environmental conditions of the different study areas. This study also agrees with Jidda and Benjamin, (2016) who isolated *Candida tropicalis, A. niger, Fusarium spp., Sclerotium cepivorum, S. cerevisiae, Rhizopus stolonifera, Scopulariopsis brevicaulis* from Monguno red and white onion bulb collected from Gamboru vegetable market in Maiduguri with the exception of *Mucor spp*. The result of this study also showed that *A. niger* had the highest percentage distribution which corresponds with Emeka (2022) who also reported a high frequency of occurrence for *Aspergillus spp* in the onion bulbs he examined. The presence of these fungi in the onion bulbs is attributable to the environmental conditions, handling and processing, storage of the onions and the quality of the onion bulbs. The fungus *A. niger* have been known to cause diseases of human and animals like ring worm and Aspergillosis (NPPC, 2006). Fungi such as *A. niger* are sources of highly potent mycotoxins which are hazardous to health and hence represent a threat to public health (Onuorah and Obika, 2015). This result is also in-line with Onuorah and Obika, (2015) and Narayana *et al*. (2007) who reported *Aspergillus spp* as the most common cause of onion spoilage. Dimkpa and Onuegbu (2010) also implicated fungi as contaminants of many agricultural commodities including onions. Al-Hindi *et al*., (2011) has isolated toxigenic fungi from spoiling fruits which further confirms the general knowledge that spoilage fungi are known to be toxigenic or pathogenic under favourable conditions (Adebayo *et al*., 2012; Dacruz *et al*., 2013; Tafinta *et al*., 2013), hence their ability to cause infections or allergies. For instance, *Aspergillus spp* are known to produce several toxic metabolites including malformis, naphthopyrones (Adebayo *et al*., 2012; Al-Hindi *et al*., 2011; Wells *et al*., 1975) as well as Ochratoxins (OTA), a mycotoxin which is considered to be a dangerous toxin to human and animal health worldwide (Petzinger and Weidenbach, 2002) and other mycotoxins that are known to be hepatocarcinogenic and nephrogenic in nature (Ajay *et al*., 2011).

Consumption of Onions contaminated by some of these spoilage fungi will not lead to loss of agricultural produce, it can also lead to toxic effect in humans that may result to death.

Conclusion

Bulb rotting caused by fungi is one of the major reasons for storage losses of onions in Nigeria as well as other countries in the world. This research revealed that different fungal genera were associated with rotten onion bulbs collected from different markets in Awka metropolis. Six fungal genera were implicated as major causal agents of rotting onion bulbs including *Fusarium spp, Mucor spp, Aspergillus niger, Sacchromyces spp, Rhizopus spp* and *Candida tropicalis*. Some of these organisms involved can be toxigenic and harmful to humans.

Acknowledgement

We are sincerely grateful to the head of Zoology Department for granting us permission to conduct this study in the departmental laboratory and also to the laboratory technologists for their assistance and for providing all needed apparatus for the laboratory procedure.

Conflict of Interest

No conflict of interest was declared by the authors

Source of Funding

None was received for this study

References

Adebayo LO, Diyaolu SA (2003). Mycology and spoilage of retail cashew nuts. *African Journal of Biotechnology*, 2(10): 369-373.

Adebayo-Tayo B, Odu NN, Esen CU, Okon IO (2012). Microorganisms associated with spoilage of stored vegetables in Uyo metropolis, Akwa-Ibom state, Nigeria. *Natural Science*, 10: 23-32.

Adongo BA, Kwoseh CK, Moses E (2015). Storage rot fungi and seed borne pathogens of onion. *Journal of Science and Technology*, 35(2): 13-21.

Ajay KG, Sushil S, Shubuhi A, Rehkah B (2011). Diversity, pathogenicity and toxicology of *A. niger*: An important spoilage fungi. *Research Journal of Microbiology*, 6(3): 270-280.

Al-Hindi RRA, Al-Najada AR, Mohammed SA (2011). Isolation and identification of some fruit spoilage fungi: screening of plant cell wall degrading enzymes. *African Journal of Microbiology Research*, 5(4): 443-448.

Ara MAH, Khatun ML, Ashrafuzzaman M (2008). Fungi causing rot at storage and market. *Journal of Bangladesh Agricultural University*, 6(2): 245-251.

Benkeblia N (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Food Science and Technology*, 37(2): 263-268.

Cheesbrough M (2006). District laboratory practice in tropical countries. Cambridge university press. United States of America. Pp442.

Corzo-Martinez M, Corzo N, Villamiel M (2007). Biological properties of onion and garlic. *Trends Food Science Technology*, 18: 609-625.

Dacruz-Cabral L, Pinto VE, Patriarca A (2013). Application of plant derived compounds to control fungal spoilage and

mycotoxin roduction in foods. *International Journal of Food Microbiology*, 166(1): 1-14.

Dimkpa SON, Onuegbu BA (2010). Mycoflora of copra and effect of bringing on some properties of copra in Nigeria. *Agriculture and Biology Journal of North America*, 1(3): 391-394.

Dorrigiv M, Zareiyan A, Hosseinzadeh H (2021). Onion (Allium cepa) and its main constituents as antidotes or protective agents against natural or chemical toxicities: a comprehensive review. *Iranian Journal of Pharmaceutical Research* 20(1): 3-26.

Emeka CPO (2022). Fungal diseases affecting onion (*Allium cepa L.*) grown under irrigation scheme in 2012 and 2013. Available on https://ssrn.com/abstract=3996257. Accessed on April 24, 2023.

Emengini JE, Igwe PC, Idhoko K (2014). Development of real estate decision support system for Awka, Anambra state, Nigeria. *International Journal of Science and Technology*, 4(8): 19-22.

Jidda MB, Benjamin F (2016). Identification of fungi associated with storage bulb rot and seed of onion (*Allium cepa L.*) in Maiduguri, Northeastern Nigeria. *International Journal of Modern Botany*, 6(2): 26-30.

Menza N, Wanyoinke W, Muturi WM (2013). Prevalence of vaginal candidiasis and determination of the occurrence of *Candida* species in pregnant women attending the antenatal clinic of Thika District Hospital, Kenya. *Open Journal of Medical Microbiology*, 3(4): 1-9.

Muhammed S, Shehu K, Amusa NA (2004). Survey of the market diseases and aflatoxin contamination of fruits and vegetables in Sokoto, north-western Nigeria. *Nutrition and Food Science*, 34(2): 72-76.

Narayana KJP, Srikanth M, Vijayalakshmi M (2007). Toxic spectrum of *Aspergillus niger* causing black mold rot of onions. *Research Journal of Microbiology*, 2(11): 881-884.

National Population Commission Census. (2006). Federal republic of Nigeria. Nigeria: The federal government printers. Pp64.

Nnadi NE, Carter DA. (2021). Climate change and the emergence of fungal pathogens. PLoS Pathog. 17(4):e1009503.

Norman JC (1992). *Tropical vegetable crops*. Ilfracombe publishing. Great Britain. Pp300.

Ole H, Torben L, Christensen LP, Ulla K, Shakuntala HT (2004). Contents of iron, calcium, zinc and B-Carotene in commonly consumed vegetables in Bangladesh. *Journal of Food Composition and Analysis*, 17(5): 587-595.

Onuorah SC, Obika I (2015). Fungi associated with the deterioration of post-harvest onion bulbs sold in some markets in Awka, Nigeria. *Bioscience and Bioengineering*, 3(5): 90-94.

Orpin JB, Yusuf Z, Mzungu I, Orpin CA (2017). Investigation of microorganisms associated with the spoilage of onions around Dutsinma metropolis. *MOJ Biology and Medicine*, 2(4): 280-285.

Petzinger E, Weidenbach A (2002). Mycotoxins in the food chain: the role of Ochratoxins. *Livestock Production Science*, 76(3): 245-250.

Rajapakse RGAS, Edirimanna FRSP (2002). Management of bulb rot of big onion (*Allium cepa L.*) during storage using fungicides.

Annals of the Sri Lankan Department of Agriculture, 4: 319-326.

Raju K, Naik MK (2007). Survey and assessment for the post-harvest diseases of onion in north-eastern Karnataka. *Karnataka Journal of Agricultural Science*, 20(1): 164-165.

Shehu K, Muhammad S (2011). Fungi associated with storage rots of onion bulbs in Sokoto state, Nigeria. *International Journal of Modern Biology*, 1(1): 1-3.

Srinivasan D, Perumalsamy LP, Nathan S, Sures T (2002). Antimicrobial activity of certain Indian medicinal plants used in folklore medicine. *Journal of Ethnopharmacology*, 94: 217-222.

Tafinta IY, Shehu K, Abdulganiyyu H, Rabe AM, Usman A (2013). Isolation and identification of fungi associated with the spoilage of sweet orange (*Citrus sinensis)* fruits in Sokoto state. *Nigerian Journal of Basic and Applied Science*, 21(3): 193-196.

Tyson JL, Fullerton RA (2004). Effect of soil borne-inoculum on incidence of onion black mould (*Aspergillus niger*). *New Zealand Plant Protection*, 57: 138-141.

Velez L, Rodriguez RP, Cabera J (2004). Fungi associated with onion (*Allium cepa*) field in southern Puerto Rico. *Journal of Agriculture of the University of Puerto Rico*, 88(1): 55-72.

Wells JM, Cole RJ, Kirksey JW (1975). Emodin, a toxic metabolite of *Aspergillus wentii* isolated from weevil-damaged chestnuts. *Applied Microbiology*, 30(1): 26-28.