EFFECT OF GARLIC (*Allium sativum*) EXTRACT ON FUNGAL SPOILAGE AND PRESERVATION OF ONION (*Allium cepa*) BULBS.

OLURANTI, O.O.[1] and SANGOYOMI, T.E.[2]
[1]Microbiology Programme, Bowen University Iwo, Osun state.
[2]Crop Production Programme, Bowen University Iwo, Osun state.
Corresponding author; yinka.oluranti@bowen.edu.ng
Phone No: +234 7068848505, (0)9047564522

ABSTRACT
The study investigated the occurrence and virulence of some fungal pathogens causing rot of onions. Antifungal potentials of garlic extract against these fungi and its effects on shelf life were also studied. Ten percent aqueous garlic extract was used in fungal growth inhibition of artificially inoculated onion bulbs as well as whole bulb preservation experiments and sterile distilled water served as control in both experiments. Results showed that *Aspergillus niger, A. niger, Penicillium* and *Rhizopus* sp occurred in rotting onion bulbs and *Aspergillus niger* was found to be the most virulent, having a mean rot diameter of 1.94mm, followed by *A. flavus* (0.42 mm), *Rhizopus* sp (0.15 mm), while *Penicillium* sp was the least virulent in causing rot in the bulbs, with 0.12 mm. Onion bulbs inoculated with blank agar disc showed no rot symptoms. There were significant differences between the control and treated samples at $p<0.05$. Garlic extract was effective in inhibiting mycelial growth of all fungal pathogens in the pretreated onion bulbs (*Aspergillus niger* (2.84 mm), *Aspergillus flavus* (1.61 mm), *Penicillium* (0.48 mm) and *Rhizopus* spp (0.54 mm) and were significantly lower than control at $p<0.05$. Whole onion bulbs (treated and control) remained healthy without any sign of rot until 4 weeks after storage however, mean rot reduction of 58% was observed in onion bulbs treated with 10% garlic extract at 9 weeks after storage. The untreated (control) samples developed rot symptoms after four weeks of storage. Garlic extract could be used to elongate shelf life of onions due to its antifungal and preservative potentials.

KEYWORDS: Onion bulbs, fungi, rot, garlic extract, preservation.

INTRODUCTION
Onion is a popular vegetable and the most widely cultivated species of the genus *Allium* (Block, 2010). Onion is used all year round and of great economic importance to the society. It

is one of the common perishable crops because they contain high moisture, have papery skin and can absorb moisture easily, becoming mushy. The problem has become complicated due to lack of or poor storage facilities. They are to be stored in cool (25 to 32°C), dry, dark and well-ventilated area such as wire mesh cabinets. The most commonly stored part of onion is the bulb and it is highly vulnerable to storage rot caused by microbes especially fungi (Tanaka, 1991). This explains why onions are cheap at a particular period of time, and are scarce and expensive at another. Several researchers have investigated the microorganisms responsible for onion spoilage during storage (Yurgel *et al.,* 2018). Fungal pathogens implicated in onion bulb rot include *Aspergillus niger, A. niger, Penicillium* sp., *Rhizopus stolonifer* and *Fusarium oxysporum* (Zlata *et al.,* 2008; Shehu and Muhammad, 2012; Adongo *et al.,* 2015). These have been managed using different methods including the use of synthetic fungicides (Kumar *et al.,* 2015), plant extracts (Ahmed *et al.,* 2017). It is therefore important to proffer solution to onion rot problem, as this can affect national economy when there is decrease in the economic value of the crop. Health of consumers is also affected leading to various diseases ranging from mild to chronic illnesses.

Garlic (*Allium sativum*) like onion is another species of the genus *Allium.* It is closely related to onions, and is considered as one of the richest source of total phenolic compounds. The organosulfur compounds contribute to its bioactive properties (Martins *et al.,* 2020). The medicinal compounds account for its use for both preventive and curative purposes. The antimicrobial activity against certain microbes including pathogenic ones has been investigated. Ho66wever, information on the biological control of microbes attacking onions and preservation or elongation of their shelf life is scarce. The objectives of this study are to investigate the virulence of four rot-causing fungal pathogens of onions; the fungitoxic effect of garlic extract as well as shelf life elongation of onion bulbs.

MATERIALS AND METHODS

Sample collection

Freshly harvested medium-sized onion bulbs and garlic cloves were purchased from Oja-oba market in Iwo, Osun State (Latitude: 7° 38' 6.97" N Longitude: 4° 10' 53.62" E). Both samples were cleaned and arranged on the laboratory bench.

Determination of bulb size

An average of 10 bulbs were picked at random and were measured. Bulb diameter was determined using UNITED Vernier calipers.

Disinfection of samples

Onion bulb samples were surface-sterilized by swirling in a bowl of 10% Sodium hypochlorite (3.5w/v) for 2 minutes and rinsed in 2 batches of sterile distilled water for 1 minute each. The bulbs were then placed on double-layered sterile paper towels under the laminar flow cabinet to dry.

Preparation of aqueous garlic extract

Garlic cloves were separated and peeled. Ten percent (10%) garlic extract was prepared by crushing 100 grams of garlic in 900 ml of sterile water using a laboratory blender, and sieved using 1.0 mm aperture sieve.

Pathogenicity test

Pathogenicity of fungal isolates was carried out using the method of Onuorah and Obika (2015) by aseptically inoculating their pure cultures (*Aspergillus flavus*, *A. niger, Penicillium* and *Rhizopus* spp) into fresh and healthy bulbs using 4 mm cork borer. The holes were plugged with blank agar discs. Wounds were sealed with petroleum jelly. Onion bulbs used as control were inoculated with blank agar discs. Inoculated bulbs were left in the incubating cupboard under close monitoring for spoilage signs. The bulbs were assessed for rot development by cutting through the points of inoculation after 7 days. When rots were present, the pathogens were re-isolated and compared with the original isolates.

Determination of effect of garlic extract on rot development

The fresh bulbs were surface-sterilized in 10% Sodium hypochlorite and bored with sterile cork-borer to a depth of 5 mm. Removed plugs were brought out into a sterile beaker. The bored holes were flooded with 0.5 ml garlic extract and inoculated with agar discs containing pure cultures of *Aspergillus niger, A. flavus, Penicillium* sp and *Rhizopus* sp using sterile inoculating needle. The holes were plugged with blank agar discs. Wounds were sealed with petroleum jelly. Onion bulbs used as control were flooded with sterile distilled water and inoculated as in the test bulbs. Sterile plugs were inserted to block the holes and then sealed with petroleum jelly (Vaseline) in both test and control trials. The bulbs were kept in incubating cupboards using complete randomized design (CRD). Three (3) bulbs were inoculated per replicate and four (4) replicates per isolate. Thermometers were placed in each hood to monitor the temperature. Each group has the different replicates which were kept in separate hoods.

Determination of effect of garlic extract on onion preservation

Onion bulbs used for this experiment were surface-sterilized using10% hypochlorite solution and rinsed in 2 batches of sterile water. Five onion bulbs per replicate were arranged in net bags and dipped in 10% garlic extract for 5 minutes. The treatment was replicated four times. Control bulbs were dipped in sterile distilled water. All bulbs were allowed to dry on double layer paper towel, arranged in well ventilated sieves and stored on laboratory shelves for eight weeks using complete randomized design.

Statistical analysis

All data recorded were subjected to statistical analysis using SPSS statistic software to identify significant differences in pathogenic effects, diameter of onion rot, botanical treatment effects. ANOVA was performed at 0.05 level of probability using LSD and Multiple Comparison-Post Hoc test.

RESULTS

The fungi isolated from the rotting onion bulbs in storage include *Aspergillus niger, A. flavus, Penicillium* sp, and *Rhizopus* sp. *A. niger* and *Penicillium* sp had the highest level of occurrence with 45% and 11% frequency respectively (Table 1). The four fungal pathogens when inoculated into healthy-looking onion bulbs produced rot symptoms (Plate 1). The different pathogens showed different levels of virulence with *A. niger* being the most virulent causing rot diameter of 1.94 mm and was significantly higher than the rest of the fungal pathogens. *Penicillium* sp caused the least rot diameter of 0.12 mm but was not significantly different from *Rhizopus* sp (0.15 mm). *A. flavus* has a higher effect and is also significantly different from *Penicillium* or *Rhizopus* sp (Table 2). Effect of garlic extract pretreatment on rot development in onion bulbs inoculated with four rot-causing fungi showed significant difference between treated and untreated onion bulbs. Pretreatment of onion bulbs with 10% garlic extract completely inhibited mycelial growth of *A. flavus, Penicillium* sp and *Rhizopus* sp (0.00 mm). Although *A. niger* showed 1.22 mm mycelial growth but was not significantly different from *A. flavus, Penicillium* sp and *Rhizopus* sp (Table 3).

Rot severity in onion bulbs dipped in 10% aqueous garlic extract was significantly lower than the control bulbs from 5 weeks after storage (5 WAS) to the end of storage period (9 WAS). There were no rot symptoms observed on both treated and control bulbs from 1 WAS to 4 WAS but from 5 (1.00) and

Table 1: Fungal pathogens isolated from rot onion bulbs in storage and frequency of occurrence

Pathogen	Frequency of occurrence (%)
Aspergillus niger	44.60
Aspergillus flavus	25.90
Penicillium sp	11.20
Rhizopus sp	18.30

* 55- Onion bulbs used for isolation from each ecological zone.

Table 2: Virulence of four rot-causing fungi

Pathogen	Mean of rot diameter
Aspergillus flavus	0.42b
Aspergillus niger	1.94a
Penicillium species	0.12c
Rhizopus species	0.15c

Different letters in the column indicate significant differences ($p<0.05$)

Table 3: Effect of garlic extract on rot development in onion bulbs inoculated with four rot-causing fungi

Pathogen	Control	Treatment
Aspergillus flavus	1.61a	0.00b
Aspergillus niger	2.84a	1.22b
Penicillium species	0.48a	0.00b
Rhizopus species	0.54a	0.00b

Different letters in the row indicate significant differences ($p<0.05$)

Table 4: Effect of garlic extract treatment on whole onion bulb preservation

Week	Average Number of rot onion	
	Control	Treatment
Week 1	0a	0a
Week 2	0a	0a
Week 3	0a	0a
Week 4	0a	0a
Week 5	1a	0b
Week 6	1a	0b
Week 7	1.75a	0.50b
Week 8	2.50a	1b
Week 9	3a	1.75b

Different letters in the row indicate significant differences ($p<0.05$)

Plate 1: Inoculated onion bulb with rot disease symptom

6 (1.00) WAS, control (untreated) began to show rot symptoms with no rot symptoms on treated onion bulbs. From 7 WAS to the end of the storage period, rot symptoms were observed on treated bulbs but were significantly lower than the control bulbs. At 7 WAS, mean rot incidence for control bulb was 1.75 and was significantly lower than treated bulbs with 0.50 mean rot incidence. The same trend was observed at 8 WAS and 9 WAS respectively (Table 4).

DISCUSSION

Isolation of *Aspergillus niger, A. flavus, Penicillium* sp, and *Rhizopus* sp from rotting onion bulb samples in this study corresponds with the findings of Orpin *et al.* (2017) who isolated *Aspergillus* spp., *Mucor* and yeast spp. from onion bulbs and Adongo *et al.* (2015) that also isolated *Aspergillus niger, A. flavus, Penicillium* sp, *Rhizopus stolonifer* and *Fusarium oxysporum* from rotten onion bulbs obtained from four major markets in Kumasi, Ghana. *A. niger* has been reported to be a recurrent fungal pathogen of onions causing rot during storage. *Aspergillus* species are usually predominant as contaminants in foods and surfaces. They are the ubiquitous fungi that contaminate foods and produce mycotoxins. Though there are xerophilic fungi that have the ability to grow and complete their life cycles under reduced water activity on dried substrates or in high levels of sugar and salts, *Aspergillus* and *Penicillium* species are among the food spoilage fungi that are referred to as 'moderate xerophiles' (Hocking, 2014) and are able to grow also in onions with relatively high moisture content. According to El-Nagerabi and Ahmed (2003), *A. niger* invades the dry scales and the other dead tissues of onions though it is avirulent to living onions.

During onion storage, a percentage of about 15% losses occurs (Deshi *et al.,* 2014, Orpin *et al*., 2017). Similar result was reported for spring onion during transportation. Association of foodborne fungi with stored foods usually result in serious economic loss(es) due to spoilage. These fungi, especially moulds produce mycotoxins that are implicated in several diseases in humans (Singh *et al.,* 2012). This indicates that the fungi are health-implicating.

High frequency of occurrence of *A. niger* in this study agrees with the views of Shehu and Muhammad (2012); Samuel and Ifeanyi (2015); Abdulsalam *et al.* (2015) and Sani *et al.* (2018) that reported *Aspergillus* spp to be the fungi with the highest frequency of occurrence amongst all fungal isolates.

The virulence of each of the isolated fungi as determined by rot diameters indicates that various organisms caused different levels of damage. For example, *A. niger* was the most virulent of the fungal pathogens causing the highest level of damage on the onion bulbs and this was followed by *A. flavus*, *Rhizopus* sp. with the least level of damage caused by *Penicillium* sp**.** Abd-Alla *et al.* (2011) studied the virulence of some bacterial isolates obtained from rotten onion bulbs through production and secretion of enzymes; and molecular analysis.

Aqueous extract of *Allium sativum* effectively inhibited mycelial growth of *Aspergillus niger, Aspergillus flavus, Penicillium* sp, and *Rhizopus* sp. both *In vitro* and *In vivo*. In studies carried out by Jaber and Al Mossawi (2007) and Abiy and Berhe (2016), garlic demonstrated strong inhibitory activities against bacteria, fungi, viruses, parasites and protozoan. The allicin content of garlic is known to contribute to its antifungal activities as it reacts with thiol groups of various enzymes such as RNA polymerase (Wallock-Richards *et al.,* 2014), and alter the necessary enzyme metabolic activity involved in the virulence of the pathogen (Ankri and Mirelman, 1999). Elshahawy and Saied (2019) also reported the effective treatment of rot disease in onion bulbs and garlic cloves using oils obtained from the plants as well as their powder. They also reported that *Allium* waste obtained from onion and garlic stimulated sclerotial germination and reduced rot incidence in the two crops. Both treatments (*Allium* oil and waste) proved to be the most effective treatments, which reduced disease incidence by 80% and 78% in garlic and onion respectively. Hence, garlic is a good antimicrobial agent.

In the whole bulb experiment, the ability of garlic extract to elongate onion shelf life was confirmed by the significant reduction in rot observed in onion dipped in 10% garlic extract over a period of 9 weeks of storage. Both control and treated groups of samples were intact and there was no rotting or any sign of distress on the samples in the first four weeks. The significant differences observed on the treated and untreated onion samples from five weeks after storage was similar to the observation of Adekalu *et al.* (2009) who reported the preservative activity of garlic (*Allium sativum*) and *Eugenia aromatic* on fresh tomato puree. Also, Udo *et al.* (2001) gave the report that garlic can be used to prevent rot in yam and potato in store. Al-Dehylaimy and Barakat (1971) also disclosed that it has been used as preservative in certain foods such as sausages and tomatoes. Similar observation was made by Olaniran *et al.* (2019) in the preservation of unpasteurized cashew juice using garlic-ginger filtrate.

Among some other botanicals, garlic was discovered a promising biopesticide

(Tafadzwa *et al.,* (2016) which can compete effectively with chemical pesticides without residual effect. Preparation of crude extract from plants for disease control is relatively cheap and can easily be practiced at household levels. It is also a safer alternative to the hazardous conventional fungicides.

Garlic is known to be highly nutritional and medicinal and have been used for prevention and treatment of different diseases (Harold, 2004). It was also discovered to contain antioxidants which protect cell damage and aging. According to Bayan *et al.* (2014) and several other authors, garlic is known to possess hypoglycemic and antihypertensive potentials. Therefore, health benefits of garlic cannot be overemphasized. Also, garlic is readily available everywhere especially as raw, and sometimes as aqueous extract preparations. They can be cheaply purchased from both local markets and supermarkets. It lasts long in storage and it is a natural spice in many soups and dishes.

CONCLUSION

Aspergillus niger, A. flavus, Penicillium sp, *Rhizopus* sp, *A. nidulans* and *Mucor* sp. are important fungal pathogens causing rot of onion bulbs. Out of the first four tested pathogens, *A. niger* was the most virulent while *Penicillium* sp was the least. The study established the pathogenicity of the tested fungal isolates in onion bulbs. The antifungal and preservative ability of garlic extract was also confirmed in this research. Onion rot in storage can be reduced by garlic extract treatment. Garlic is widely available and can be recommended as food preservative without adverse effect on human health and environmental safety.

RECOMMENDATION

Further research may be carried out to evaluate the effect of multiple treatment regimes on onion shelf-life elongation.

ACKNOWLEDGEMENTS

The contributions of the following people are highly appreciated; Mrs. B. O. Atobatele and Mrs O.T. Akinola (Microbiology Programme), Mrs Omoyajowo (Mathematics and Statistics Programme) and Dr. V. I. Esan (Agriculture Programme), Bowen University, Iwo, Nigeria.

REFERENCES

Abiy, E., and Berhe, A. (2016). Antibacterial Effect of garlic (*Allium sativum*) against clinical isolates of *Staphylococcus. aureus* and *E. coli* from patients attending Hawassa Referral Hospital. Ethiopia. *J. of Infectious Diseases and Treatment,* **2** (2): 44- 53. doi: 10.21767/2472-

1093.100023

Abd-Alla, M.H. and Bashandy, S.R., Ratering, S. and Schnell, S. (2011). First Report of soft rot of onion bulbs in storage caused by *Pseudomonas aeruginosa* in Egypt. *J. of Plant Interactions,* **6**(4): 229-238

Abdulsalam, A. A., Zakan, B.G., Ghimbekujwo, I.B., Channya, F. K. and Bristone, B. (2015). Isolation and Control of fungi associated with neck rot disease of onions (*Allium cepa* L.) in Bama, Borno state. *Global J. of Biology. Agriculture and Health Scs.,* **4**:35-39

Adekalu, O. A., Olatunde, I. G., Echendu, B. M., Adepoju, T. C. and Fajemisin, O.O. (2009). Antimicrobial and preservative activities of *Allium sativum* and *Eugenia aromatic* on fresh tomato puree. *African Journal of Agricultural Research,* **4**(2): 139-140

Adongo, B.A., Kwoseh, C. K. and Moses E. (2015). Storage rot fungi and seed-borne pathogens of onion. *Journal of Sc. And Technology (Ghana),* **35**(2):13-21

Ahmed, A. A. and Mustapha A. (2017). Isolation and Management of fungal pathogens associated with Neck rot disease of onions (*Allium cepa* L.) in Maiduguri, Borno state. *Chem Res J,* **2**(4): 73-77

Al-Dehylaimy, K.S. and Barakat, M. M. (1971). Antimicrobial and preservative activity of garlic on fresh ground meat. *J. Sci. Food Agric,* **22**: 96-98

Ankri, S., and Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. *Microbes Infect,* **1**(2): 125-129

Block, E. (2010). Royal society of chemistry. *Garlic and other Alliums: the love and the science,* ISBN 978-0-85404-190-9.

El-Nagerabi, A. F. and Ahmed, A. H..M. (2003) Storability of onion bulbs contaminated by *Aspergillus niger* mould. *Phytoparasitica* 31(5): 515-523

Elshahawy, I. E., and Saied, N. M. (2019). Reduction of *Stromatinia cepivora* inocula and control of white rot disease in onion and garlic crops by repeated soil applications with sclerotial germination stimulants. *Elsevier,* **5** (1): e01168

Deshi, S. N., Wonang, D.L. and Dafur, B. S. (2014) Control of rots and spoilage of agricultural products: a review. *International Letters of Natural Sciences* 18:63-72

Hocking, A. D. (2014) Spoilage Problems. Problems caused by fungi. *Encyclopedia of Food Microbiology (Second Edition) pgs 471-481*

Kumar, V., Neera, Sharma, S. and Sagar N. A. (2015). Post harvest management of Fungal Diseases in onion- A Review. *Int. J. of Current Microbio. And Appl. Scs,* **4** (6): 737-752

Martins, N., Petropoulos, S., Ferreira, Isabel C. F. R. (2020). Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: A review,

Olaniran, A.F., Okolie C., Abu H. E., Afolabi, R.O. and Owolabi, A. (2019). Preservative effect of garlic-ginger, sodium benzoate and ascorbic acid in unpasteurized cashew apple juice. *Asian J. of Sc. Res,* **12**(3): 414-420

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

Orpin, J. B., Yusuf, Z., Mzungu, I. and Orpin, C. A. (2017). Investigation of microorganisms associated with the spoilage of onions around dutsinma metropolis. *MOJ Biol Med***,** **2**(4):280-285

Samuel, O. and Ifeanyi, O. (2015). Fungi associated with the deterioration of post- harvest onion bulb sold in some market in Akwa, Nigeria. *Bioengineering and Biosc.* **3** (5): 90-94

Sani, I. H., Sulaiman I., Illiyosu Z. and Abdulsalam, U.S. (2018). Antioxidant potential of *Phoenix dactylifera* Linn extract and its effects on calcium channel antagonist in the treatment of withdrawal syndrome in morphine dependent rats. *Trop J. Nat Pro. Res,* **2**(7): 309-313

Shehu, K. and Muhammad, S. (2012). Fungi associated with the storage rots of onion bulb in Sokoto, Nigeria. *International Journal of Modern Botany.* **1**(1):1-3.

Singh, A., Wang, H., Silva, L.C., Na C., Prieto, M. and Anthony H. (2012) Methylation of glycosylated sphingolipid modulates membrane lipid topography and pathogenicity of *Cryptococcus neoformans*. *Cellular Microbiology* 14 (4): 500-516

Tanaka, K. (1991). Studies on the black mould disease of onion bulbs caused by *Aspergillus niger* van Tieghem. *Bulletin of Faculty of Agriculture. Saga University,* **70**: 1-54

Udo, S.E., Madunagu, B. E., Isemun C. D. (2001). Inhibition of growth and sporulation of fungi pathogens on sweet potato and yam by garlic extracts. *Niger. J. Bot,* **14**: 35-39

Wallock-Richards, D., Doherty C. J., Doherty L., Clarke D.J., Place, M., Govan, J.R.W. and Campopiano D. J. (2014). Garlic Revisited: Antimicrobial Activity of Allicin-containing garlic extracts against *Burkholderia ceparia* complex *PLoS ONE* **9**(12): e112726. doi: 10.1371/journal.pone. 0112726.PMID:25438250; PMCID:PMC4249831.

Yurgel, S. N., Abey, L. and Loomer, N. (2018). Microbial Communities Associated with Storage Onion. *Phytobiomes* **2**: 35-41

Zlata, D. Klokocar.Smith., Jelena T. Levic., Stevan, N. Masirevic., Jelina M. Grozdanovic-Varga., Mirjana A. Vasic. and Svjetlana, R. Aleksic (2008). *Fusarium* rot of onion and possible use of bioproduct. *Proc. Nat. Sci. Matica Srpska Novi Sad,* **114**, 135-148