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Fungi associated with onion (*Allium cepa* L.) bulb rot and the impact of storage containers on the occurrence of the fungi in market centers, Tamale, Ghana

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Abstract

The onion bulb rot which is caused by a number of fungi pathogens is considered one of the most important factors leading to high postharvest loss in onions. Studies were conducted to determine the incidence of onion bulb rot, identify the associated fungi and evaluate the impact of storage containers on the incidence of the disease. Thirty (30) rotten onion bulbs var. 'Bawku Red' samples were collected from two markets in Tamale and studied in the Spanish laboratory, Faculty of Agriculture, University for Development Studies, Nyankpala. The results showed that the onion bulb rot was caused by five fungi species including *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., *Rhizopus stolonifer* and *Fusarium oxysporum*. *A. niger* had the highest percentage occurrence of 30.66% and 25.66% for the Tamale Central and Aboabo markets, respectively. The pathogenicity test conducted showed that *A. niger* and *R. stolonifer* were the most pathogenic species. Generally, black mould (*A. niger*), blue mould (*Penicillium* sp.), soft rot (*R. stolonifer*) and basal plate rot (*F. oxysporum*) were the commonest diseases associated with onion bulbs in the Tamale markets. The most encountered disease was black mould while the least was soft rot. The incidence of onion bulb rot was significantly ($P < 0.05$) higher in onions that were stored in sacks compared with those stored in baskets and pans. In order to reduce storage rot of onion bulbs, there is the need to encourage the use of pans and baskets for storing onion bulbs in the markets.
Key words: Fungi pathogens, black mould, blue mould, soft rot, basal plate rot, onion bulbs

Introduction

Onion (*Allium cepa*) is a vegetable crop which is widely used in many parts of the world to add flavor to foods, for seasoning foods and for medicinal purposes (van der Meer, 1997). Medicinally, onion has been used for the prevention of cardiovascular disorders, reduction in blood levels, osteoporosis, stomach ulcers, inhibition of platelets-mediated thrombosis, treatment of fever, common cold and sore throat (Abbas, 2005). In many Ghanaian homes, onions are used in the preparation of various kinds of foods and for seasoning meat and fish (SRD/ MoFA, 2002). In Ghana, the Northern and Upper East regions are popular areas for commercial onion production (Obeng-Ofori *et al*., 2007). Seasonal production, unfavourable weather conditions, insufficient access to inputs and diseases and pest problems contribute to the low production and scarcity of onions in Ghana,

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especially during the off-season (SRID/MoFA, 2002). Microorganisms particularly fungi cause serious postharvest losses in many vegetables including onions. Some species of fungi that cause losses in onions at storage includes the *Aspergillus* spp., *Botrytis* spp, *Alternaria* spp. and the *Peronospora* spp**.** (Adongo *et al.*, 2015). *A. niger* is responsible for the black mould disease and it causes significant amount of postharvest losses in onions all over the world (Cabera *et al*., 2004).
The presence of fungi especially *A. niger* on onion bulbs under warm environmental conditions results in 30 - 80% loss or damage (Raju and Naik, 2007). Smolinska (2000) reported that the rapid deterioration of onion bulbs may occur due to physical influences, actions of microorganisms or a combination of these factors. The microorganisms causing onion bulbs rot can be transferred through direct contact with infected or damaged onions and therefore causing further spread of the disease. Association of *A. niger* with onion bulbs produced in hot climates and their transmission from soil and naturally-contaminated bulbs to onion seedlings cause 30 - 80% loss or spoilage of onion bulbs (Ifeanyi, 2005). Fungal infections can also increase the chances of contamination by mycotoxins which are hazardous to human and animal health (Kumar *et al*., 2015).

Storage rots of onion bulbs decrease the quantity and market value of bulbs (Shehu, 2001; Adongo, 2005). During storage, suitable environmental conditions must be provided to maintain the quality of the onions bulbs. Storage losses are a function of storage environment as well as the condition and cultural practices used during the growing season. Proper control of the climatic conditions in the storage environment can significantly extend the shelf-life of stored onion bulbs (Alderman and Lacy, 1984). The effect of storage containers on the incidence of onion bulb rot has also been reported. Srinivasan and Shanmugam (2006) evaluated the efficacy of six types of containers/methods viz., jute gunny bags, polyethene lined gunny bag with perforations, bamboo basket, bamboo bins, wooden rake and hanging method in reducing the spoilage in stored onions. They reported that containers used for storage of onion bulbs showed significant influence on the incidence and development of *Aspergillus nige*r rot. The most affected bulbs were found in onions stored in cold room (6°C) followed by Free floor and Bamboo basket. The lowest infected bulbs were found in onions stored in dried sands followed by net-bag and jute bag. Thus, the fungal diseases in storage are higher in cold room (6°C) than net-bag or jute bag (Srinivasan and Shanmugam, 2006). In other studies, storage containers had significant effect on seed quality during storage. This effect resulted from the moisture that is accumulated within the containers under study (Kalyanrao *et al*., 2018). The objectives of this study were to determine the incidence of fungi in stored onions at two markets centers in the Tamale and also assess the impact of storage containers on the incidence of onion bulb rot.

Materials and Methods

Collection of onion samples

The samples used for the study were collected from two major markets (Aboabo and Tamale Central market) in the Tamale (9°25 N, 0°51 West and 173 m above sea level) Metropolis for laboratory analysis.

Thirty (30) onion samples were collected from different storage containers (Plate 1) at the two markets. The samples were collected in the month of April 2018 when the average day and night temperatures were 35°C and 25°C, respectively. Fifteen (15) samples collected from Aboabo and fifteen (15) from the Tamale Central markets. The samples were packed in paper bags and transported to the laboratory for analysis.

Laboratory studies

The laboratory studies were carried out in the Spanish laboratory of the University for Development studies, Nyankpala campus. Tamale metropolis is located at latitude 9°25 N and longitude 0°51 West and has an elevation of approximately 173 m (568 feet).

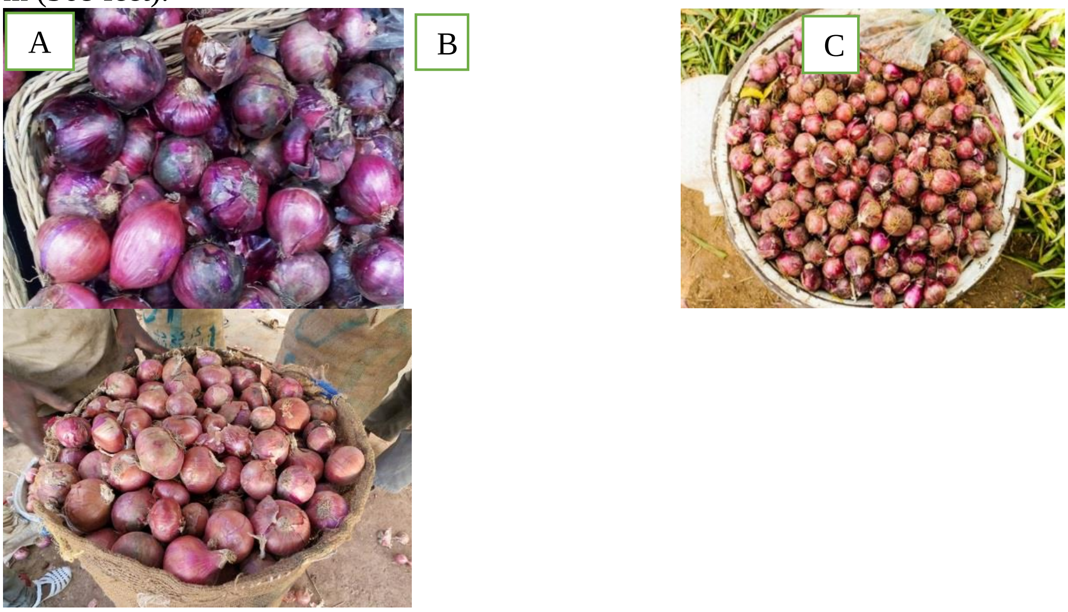

Plate 1: Samples of baskets (A), pans (B) and sacks (C) used for onion storage in the two markets

Incidence of onion bulb rot

The onion bulb samples collected from the different storage containers were observed carefully for symptoms of rot and those that showed visual symptoms of rot were counted. The percentage disease incidence (PDI) for each disease was determined as described by Raju and Naik (2007):

PDI = Number of infected bulbs/Total number of bulbs x 100

Experimental design

Completely Randomized Design (CRD) was used in the study. Thirty onion samples were collected from Aboabo and Tamale Central market. Five randomly selected slices of onion bulbs from each sample were plated equidistant on potato dextrose Agar (PDA) and replicated three times.

Preparation of culture medium

The culture medium was prepared according to the instructions of the manufacturer (Oxoid, UK). Thirty-nine grams (39 g) of PDA was dissolved in 1 L distilled water in a conical flask. The opening of the conical flask was plugged with cotton wool and covered with aluminum foil to prevent contamination, condensation and loss of moisture. The mixture was stirred using a magnetic stirrer whiles heating it on the heating mantle to obtain a homogenous mixture. The mixture was then autoclaved at 121^{o}C at pressure of 1 kg/cm^2 for 15 minutes and allowed to cool afterwards. About 20 ml of the melted PDA was poured into a sterilized petri dish in a controlled environment (lamina flow hood) and allowed to solidify. The plates were now ready to be used.

Isolation of fungi

Five (5) onion bulbs from each sample were sliced and surface sterilized in 1% sodium hypochlorite (NaOCl) solution for 3 minutes. One (1%) was used to prevent the fungi from being killed and to reduce surface contamination. The slices of onion bulbs were rinsed three times with distilled water and left to dry on sterilized filter papers. The samples were plated equidistant on Potato Dextrose Agar (PDA). The plated sliced onions were incubated at 25°C for five days. After five days of incubation, mixed cultures of various species of fungi were obtained from the plated onions. The mixed cultures were sub-cultured by transferring a loop full from each of the species on to PDA plates. The inoculated plates were incubated at 25^{o}C for five days.

Identification of fungi

The different fungi were identified based on colony characteristics, strain morphology and microscopic features as described by Klich (2002).

Determination of incidence of fungi

The incidences of fungi isolated from infected onions bulbs were determined using Potato Dextrose Agar (PDA) following the procedures described by Cotty (1994).

Pathogenicity test

The outer dry scales of healthy onions were removed and cleaned with cotton soaked in seventy percent alcohol and this was carried out aseptically. Cavities were made in the bulbs, using a 3 mm diameter sterile cork borer and the tissues plugs were pulled out. The fungal disc was inserted into the cavities and the onion plugged placed back. The wounded area was sealed with Vaseline to prevent external infections. The bulbs were incubated for 3 weeks at 28°C in the incubation chamber. Three replications were made with three bulbs for each fungus. Observation for rot development was made and the degree of pathogenicity of each fungus determined by measuring the extent of the rot (mm) using a rule.

Statistical analysis

The recorded data on various parameters under the present study were analyzed by analysis of variance using Genstat (Edition 4). The Least Significance Difference (LSD) test was used for the separation of means at 5%**.**

Results

Incidence of onion bulb rots in two markets in Tamale.

During the survey of the markets to determine the incidence of postharvest diseases of onion, five diseases including soft rot, blue mould, black mould, basal plate and basal neck rot were found (Table 2). Both markets recorded moderated incidences of the postharvest diseases (Table 1). The most common disease was black mould, followed by blue mould, then basal plate rot, neck rot and the least was soft rot.

Table 1: Incidence of postharvest diseases of onion in Aboabo and Tamale central markets

	Postharvest disease incidence (%)				
Markets	Soft rot	Black mould	Blue mould	Basal plate rot	Neck rot
Aboabo	14	31	22	16	17
Tamale Central	12	35	25	17	11
Mean	13	33	23.5	16.5	14

Occurrence of onion rot fungi species in two markets in Tamale

Five species of fungi were isolated from all the thirty samples collected from the two markets within the Tamale metropolis. These were *Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer* and *Penicillium* sp. *A. niger* recorded the highest percentage occurrence 30.66% and 25.66% for the Tamale Central and Aboabo markets respectively. *Penicillium* sp. followed with a percentage occurrence of 20.43% for the Tamale Central market and 22.90% for the Aboabo market. *F. oxysporum* followed with a percentage of 18.63% and 15.33% Tamale Central and Aboabo market respectively. *R. stolonifer* also recorded a percentage of 12.90% and 16.90% for Tamale Central and Aboabo market, respectively. However, the least fungal incidence was found in *A. flavus* with 14.35% and 14.22% in Tamale and Aboabo markets respectively. In general, there were no significant difference ($P > 0.05$) in the percentage occurrence of the individual fungi species in the two markets.

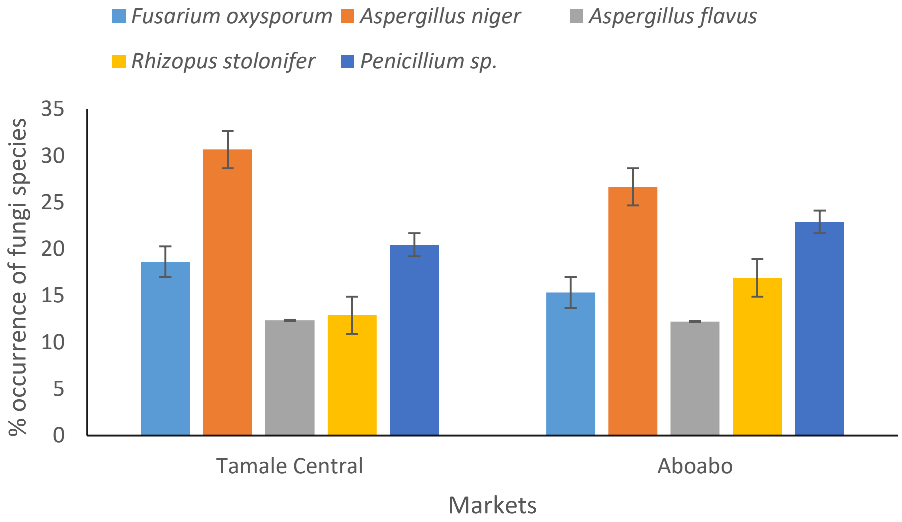

Figure 1: Percentage occurrence of fungi species from the two markets

Effect of storage containers on incidence of fungi species

Onion samples were collected from the two markets and bulbs were sampled from three (3) storage containers including jute sacks, baskets and pans. From the jute sacks, *Aspergillus niger* was the most common (37.35%) followed by *Penicillium* sp. (27.35%), *Fusarium oxysporum* (24.65%), *Aspergillus flavus* (22.19%) And *Rhizopus stolonifer* (22%). With respect to the bulbs kept in pans, *Aspergillus niger* was the commonest (24.65%) followed by *Penicillium* sp. (20.30%), *Rhizopus stolonifer* (16%), *Aspergillus flavus* (10.66%) and *Fusarium oxysporum* (10.65%). With regard to bulbs stored in baskets, *Aspergillus niger* again was the commonest (25.35%) followed by *Penicillium* sp. (17.35%), *Fusarium oxysporum* (11.30%), *Aspergillus flavus* (10%) and *Rhizopus stolonifer*, the least incidence (6.70%). There was significant difference ($P < 0.05$) between incidence of *A. niger* and *Penicillium* sp. in relation to storage containers whilst there was no significant difference ($P > 0.05$) in among *F. oxysporum*, *A. flavus* and *R. stolonifer* in relation to storage methods.

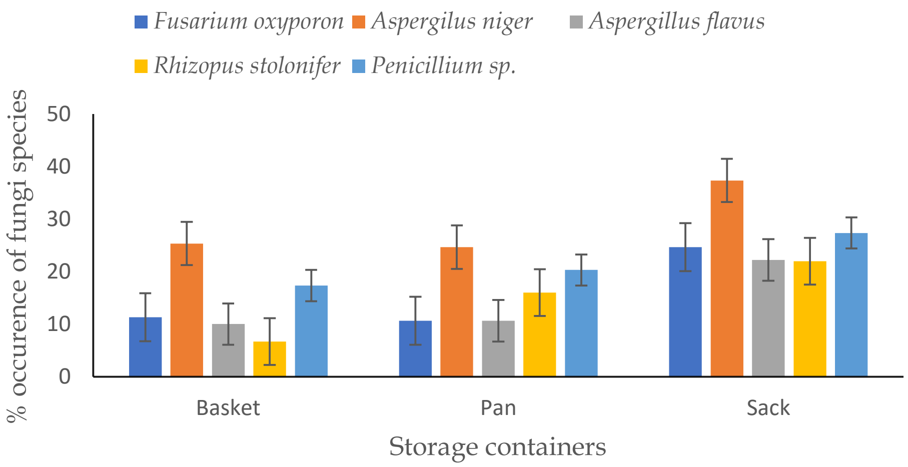

Figure 2: Percentage occurrence of fungi species based on storage containers

Assessment of fungal pathogens associated with storage rots of onion

Four genera consisting of five species of fungi were found. *A. flavus*, *A. niger*, *Fusarium oxysporum*, *Penicillium* sp. and *Rhizopus stolonifer* were isolated from the contaminated onion bulbs collected from the two main markets (Aboabo and Tamale Central markets) in the Tamale Metropolis (Table 2). *Aspergillus niger* had the highest rate of occurrence (47.23%), followed by *Penicillium* sp. (23.80%), *Fusarium oxysporum* (12.34%) and *Rhizopus stolonifer* (10.60%). *Aspergillus flavus* had the least value in terms of occurrence (6.0%).

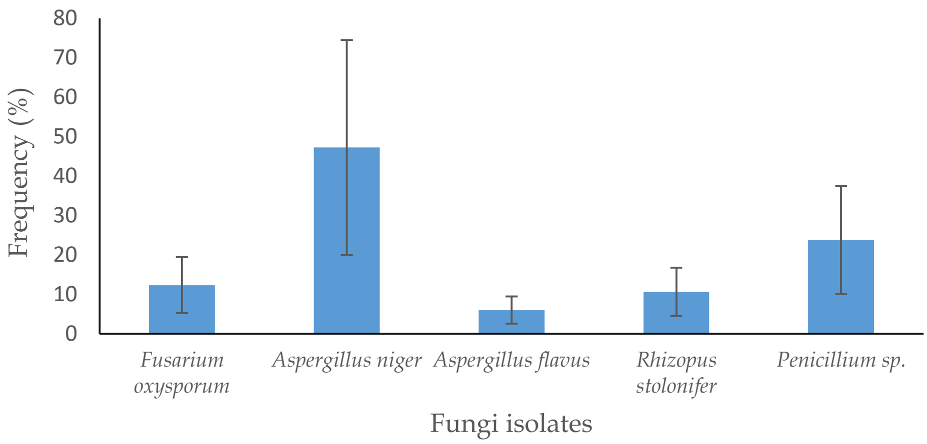

Figure 3: Percentage occurrence of isolated fungi associated with storage rot of onion bulbs in the two markets

Pathogenicity of isolated fungi on onion bulbs

Aspergillus niger produced the largest rot diameter (38 mm) followed by *Rhizopus stolonifer* (36 mm) with *Penicillium* sp. producing the least rot (14 mm).

Table 3: Pathogenicity of isolated fungi on onion bulbs determine by rot diameter after 14 days of incubation

Pathogen	Diameter of rot on onion bulbs (mm)
Aspergillus flavus	18
Aspergillus niger	38
Fusarium oxysporum	27
Penicillium sp.	14
Rhizopus stolonifer	36
Control	6

Discussion

The present study showed that black mould, neck rot, blue mould, and basal plate rot were the most important disease pathogens associated with onion bulbs stored in two markets in the city of Tamale, Ghana. The black mould, caused by *A. niger*, was the commonest disease on onion bulbs in the Tamale and Aboabo markets. Raju and Naik (2007) conducted a similar study and reported that the black mould was among the important postharvest disease of onions in Karnataka, India. The Black mould is a primary rot disease which commonly occurs when onions are stored under ambient temperature conditions (Ko *et al*., 2002).

Five fungi pathogens, including *Aspergillus flavus, A. niger, Fusarium oxysporum, Penicillium* sp. and *Rhizopus stolonifer* were isolated from the diseased onion bulbs which were collected from the Aboabo and Tamale Central markets. However, *Aspergillus niger* had the highest occurrence (47.23%). This results is similar to the findings of Ko *et al*. (2002) who reported that *Aspergillus niger* is commonly associated with onion bulbs and it is the most common fungi that causing onion bulb rot. The study also revealed *Aspergillus niger* is the fungus that causes rapid bulb rot among the pathogens found. Our results conforms to the findings of Adongo (2002) who reported that *Aspergillus niger* causes decay in onion bulbs at a very fast rate.

There were significant differences ($P < 0.05$) in the incidence of fungi pathogens recorded in onion samples from the different storage containers (sack, basket and pan). The incidence of onion bulb rot was highest in onions that were stored in sacks. It is likely that the different storage containers provided different storage conditions that influenced the mycelium growth in storage. Each of the storage containers sampled in the study produced an amount of moisture that influenced fungal growth. The basket had low incidences of all the fungi identified as compared to the other storage containers because its ability to allow adequate aeration of the bulbs. Reis and Lopes (2007) have indicated that the development of *Aspergillus niger* is enhanced under wet and cool environments.

Conclusion

The study was conducted with onion samples collected from the two major markets in the city of Tamale. The results revealed that soft rot, neck rot, black mould and blue mould were the main postharvest diseases associated with the onion bulbs in the markets. *Aspergillus flavus, A. niger, Fusarium oxysporum, Penicillium* sp. and *Rhizopus stolonifer* were identified as the fungi pathogens responsible the various onion bulb rots in the markets. *Aspergillus niger,* which was the most pathogenic, was also the commonest in both markets. The incidence of onion bulb rot was highest in onion bulbs which were stored in sacks. In order to reduce the high losses of onion bulbs which occur in the Tamale markets, storage in baskets and pans should be encouraged.

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