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Isolation And Control Of Fungi Associated With Neck Rot Disease Of Onions (*Allium Cepa* L.) In Bama, Borno State, Nigeria

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Abstract

A survey of fungi associated with the neck rot disease of onion bulbs was conducted in Bama, Borno state Nigeria. The aim of this study was to test the effect of plant extracts in the control of fungi associated with neck rot disease of *Allium cepa* in markets of Bama and environments. Four fungi; *Aspergillus niger, Botrytis aclada, Penicillium expansum* and *Aspergillus flavus* were isolated. Pathogenicity test indicated that all the isolates were pathogenic on *Allium cepa*, however, *A. niger* was the most virulent of the other test organisms with highest mean rot diameter of 40.0 mm and *P. expansum* was the least virulent with mean rot diameter of 21.00mm. *A. niger* had the highest incidence of the disease in the study area and predominant in Woloji (96) %, Gulumba (90) % and Bama (75) %. Least encountered were *P. expansum* with disease incidence of (45)% each in Bama and Gulumba and (0.00)% in Woloji. Three plant extracts were tested on the fungal isolates. Of these Plant extracts tested, Mahogany leaves extract was highly effective against the test organisms than the other plants extract and cheaper compared to the pesticides which are highly toxic and costly.

Keywords: *Aspergillus niger, Aspergillus flavus, Botrytis aclada, Pencillium expansum, Allium cepa,*

1. Introduction

Onion was originally native to Central Asia, later spread to the other parts of the world, and hence finds their way to the Egyptian trade, where they became a crucial food plants in the world (White and Zellner, 2008). They prefer light sandy soils in cool moist region. Onion is classified under the kingdom - *Plantae*, class - *Angiosperm*, order - *Asparagales*, family - *Amaryllidiaceae* and Genus - *Allium* (Thompson, 1995). Hill and Waller (1988) indicated that onions have been attacked by many diseases namely; downy mildew, purple blotch, blast and neck rot, white rot, rust, smudge, leaf spot, pink rot, and basal rot etc. Raju and Naik (2007) elaborated that onion suffers from many post harvest fungal diseases, like the soft rot, brown rot and smudge. They also reported that these infections restrict the availability of onions to both domestic and international trade. Diseases of onion include:- Neck rot, scape blight and umbel blight (Chilvers and Dutoit, 2006). White and Zellner (2008) reported that onions are vulnerable to a handful of diseases and disorders caused by of fungi, bacteria and viruses. These diseases include *Downy mildews*, onion smut, onion smudge, white rot, neck rot, soft rot and Aster yellow (White and Zellner, 2008). In storage conditions a number of fungi, including *Fusarium* sp. *Botrytis* spp. and *Aspergilus niger*, were found on diseased onion bulbs (Joon *et al*., 2001). Joon *et al*. (2001) reported that major pathogens associated with basal and neck rots were F*usarium oxysporum* and *Aspergillus* spp. or *Botrytis allii*, respectively, of which basal rot was most prevalent and damaging during storage. In Borno state most of the spoilage of fruits and vegetables are caused by Aspergillus niger, though other disease such as Botrytis plays a significant role in Allium cepa spoilage and has been a major concern to the farmers (Ibrahim, 2005).

Onion cultivation is financially attractive and most importantly an essential component of the daily food intake in every home that contributes to the nutrition. Allium cepa (onion) is important large-scale commercial crop for processing and export; it has high worldwide demand and foriegn exchange earning potential (Ibrahim, 2005). In view of that, this study aimed at testing the effect of plant extracts in the control of fungi associated with neck rot disease of *Allium cepa* in markets of Bama and environments.

2. Materials and Methods

2. 1 Sites of the Sample Collection

The onions sample for this study were collected in three district headquarters within Bama Local Government Area, namely:- Bama, Woloji (Kumshe) and Gulumba because they are the sites for onion cultivation. The samples were collected from the months of March-April (2011) normally onions were allowed to stay on the field for a period of four to five months to fully mature before they were harvested to reduce rot in stores.

2.2 Sample Collection

Three hundred onion bulbs sample were collected from markets in each of the three districts mentioned. The infected onions were separated from the healthy ones and disease incidence was estimated. The samples were kept in a polythene bags and labelled separately and were taken to the laboratory for further studies.

2.3 Estimation of Disease Incidence

Percentage disease incidence was calculated using the formula below;

$$\text{Disease Incidence} = \text{Percent}\ \frac{Number\ of\ infected\ bulbs}{Total\ number\ of\ bulbs\ collected}\ X\ 100$$

2.4 Sterilization

The inoculating needle and the cork borer were sterilized by flaming using a spirit lamp. The glass wares such as the petri dishes, the conical flasks and test-tubes were sterilized in a hot air oven at 160^{O} C for 2 hours. The table was sterilized using 70% alcohol for 30 minutes.

2.5 Preparation of Culture Media

The culture medium used in this study was Potato Dextrose Agar (PDA)

Composition:

Potatoes (peeled/sliced) ….....200 g
Dextrose ……...20 g
Agar ……..…….15 g
Distilled water ……..1000 ml

2. 5. 1 Preparation of potato dextrose agar medium

Irish potato tubers were peeled and sliced into thin pieces and boiled in 500 ml distilled water for 15 minutes. The potato infusion was decanted into a beaker and the slice discarded. In a separate beaker 500 ml of distilled water was boiled while agar was added slowly and stirred using a glass rod to avoid lump formation and dextrose was dissolve in the potato infusion. The melted agar and the potato infusion were thoroughly mixed and made to volume of 1000ml by adding distilled water. The medium thus prepared was transferred into a fresh Erlenmeyer flask, plugged with non-absorbent cotton wool. The plugs were wrapped with aluminium foil and sterilized in an autoclave at 121^{o} C for 20 minutes at 15 lbs prior sterilization; 1-2 drops of lactic acid were added to the medium to inhibit bacterial growth. Cooled sterilized medium was dispensed into sterile petri dishes and allowed to solidify awaiting inoculation.

2.6 Isolation of Organisms

The diseased portion of the onion bulbs were surface sterilized with 1% sodium hypochlorite and rinsed in sterile distilled water three times. The rinsed disease portion were cut into tiny bits and blotted dry with sterile Whatman No.1 filter papers. Thereafter, the onions were aseptically plated into 9 cm Petri dishes containing sterile solidified PDA. Distinct colonies from the isolation plates were sub cultured to obtain the pure culture.

2.7 Preparation of Slides

A small portion of the fungal colony was scooped from the plate using a sterile inoculating needle into a glass slide. One to two drops of laptophenol cotton blue was added and covered with the cover slip. The glass slide was passed over Bunsen flame to expel the air bubbles trapped in the glass. The slide was then viewed under compound microscope with x10 or x100.

2.8 Identification of fungi

Slides prepared from the individual fungal colonies were examined under the microscope to study their morphological features. The fungi identified were compared with the observed features of the colony descriptions by Chilvers and Dutoit (2006) and Robbert and Ellen (1988).

2.9 Pathogenicity Test

Five healthy onion bulbs were collected and using a sterile 5 mm cork borer, holes were made on the each bulb to make a wound. Inoculums were introduced into the wounds using a sterile inoculuting needle. The tissues were replaced and sealed with masking tape and labeled and incubated at 30^{o} C for 7 days. Constant observation was made and then re-isolated and compared with the original isolates. Controls were prepared by making the wounds aseptically on the fresh onion without inoculation. Other treatments and the incubation were the same.

2.10 Extraction of Different Plant Leaves

Three different plants leave thus, African mahogany (*Khaya senegalensis,)* Neem (*Azadiratcha indica)* and Bitterleaf (*Vernonia amygdalina)* were thoroughly washed under a running tap water, airdried separately and micronized using a hammer mill into a fine powder. Cold water extraction was obtained by adding 10, 20, 30 and 40 g of the fine powder of each leaf into a 100 ml distilled water separately in 250 ml beaker. This was left for 24 hours and subsequently filtered through four fold of sterile cheese cloth to obtain 10, 20, 30 and 40% crude aqueous extract (Taiga, 2011).

2.11 Control using leaf extracts

Ten milliliters of an appropriate dilution of each plant leaf extracts poured into 9 cm Petri dishes with solidified potato dextrose agar and drained off. Then a 3 day old colony was cut using a sterile 5 mm cork borer and placed aseptically upside down in the centre of the PDA in the plates and incubated at 30^{o} C for 7 days. Constant observation was made.

2.12 Experimental Design and Statistical Analysis

A completely Randomized Design was used as described by Gomez and Gomez (1984). The experiment was replicated 3 times and data obtained was analysed using Analysis of variance (ANOVA) and the means were separated using Least Significant Difference (LSD) at p = 0.05.

3. Results and Discussion

3.1 Isolation and Identification

During the survey of fungi associated with the neck rot disease of onions in the three districts of Bama Local Government Area, four organims were isolated and identified as *Penicillium expansum*, *Botrytis aclada*, *Aspergillus niger* and *Aspergillus flavus* (Table 1).

Table 1**:** The Results of the Organisms Isolated in the three Districts of Bama Local Government Area

	Districts		
Organism	Bama	Woloji	Gulumba
Penicillium expansum.	√	-	√
Botrytis aclada	√	√	√
Aspergillus flavus	√	√	-
Aspergillus niger	√	√	√

KEY: √= isolated, - = not isolated

3.2 Estimation of Percentage Disease Incidence

A*spergillus niger* had the highest percentage incidence of 96% in Woloji, followed by 90% in Gulumba, and Bama had the percentage incidence of 75% . Furthermore, Bama had the highest percentage incidence of 60% *Aspergillus flavus*, followed by 45% in Woloji district while it was not encountered in Gulumba. Moreso, *Botrytis aclada* had the highest percentage incidence of 75% in Gulumba, followed by 60% in Bama, and least percentage incidence of 30% was recorded in Woloji respectively (Table 2).

Table 2: The Percentage Disease Incidence in each of the three Districts of Bama Local Government Area

	Locality and percentage incidence (%)		
Isolates	Bama	Woloji	Gulumba
Penicillium expansum	45	00	45
Botrytis aclada	60	30	75
Aspergillus flavus	60	45	00
Aspergillus niger	75	96	90

3.3 Pathogenicity Test

The pathogenicity test revealed that all the isolated fungi were pathogenic on onion bulbs, however, *Aspergillus niger* was the most virulent when compared to the remaining three organisms because it had the highest mean rot diameter of 40.00 mm, followed by *Botrytis aclada* with mean rot diameter of 37.30 mm, *Aspergillus flavus* with mean rot of 28.40 mm and *Penicillium expansum* with mean rot diameter of 21.00 mm being the least pathogenic (Table 3).

Table 3: The Results of Pathogenicity Test of Isolated Organisms Incubated for 7 Days.

Isolates	Rot diameter (mm)
Aspergillus niger	40.00
Botrytis aclada	37.30
Aspergillus flavus	28.40
Penicilium expansum	21.00

3.4 Effects leaf extracts on Fungal Isolates

Higher concentrations of 30-40% of the plant extracts control the vegetative growth of the organisms (Table 4). The studies indicated that Mahogany leaf extract was most effective in controlling the vegetative growth of fungi as depicted in (Table 5).

Table 4: The Effect of Different Concentrations of Plant Extracts on the Organisms

Concentration (%)	Bitter leaf	Neem	Mahogany
10	29.42	20.33	9.75
20	28.33	20.08	9.57
30	22.17	12.79	8.63
40	25.08	10.42	4.49
50(cont.)	34.50	28.75	30.83
Mean	27.90	18.48	12.65
LSD	3.67	2.69	1.03
P- Value	0.00029**	0.0001**	0.0001**

Key: ** = Highly Significant at (p=0.01), Cont. = Control

Table 5: The Effect of the Different Plant Extracts on the Fungi Associated with Onion Bulbs

Organisms	Bitter leaf	Neem	Mahogany
Aspergillus niger	28.20	20.40	12.83
Aspergillus flavus	29.33	19.30	13.63
Botrytis aclada	26.27	17.47	12.39
Penicillium expansum	27.80	16.73	11.77
Mean	23.4	18.48	12.29
LSD	3.28	2.41	0.93
P- value	0.00029**	0.0001**	0.0001**

Key: ** Highly Significant at (p= 0.01)

Discussion

The study was carried out in three localities, namely, Gulumba, Bama and Woloji. Four (4) organisms were isolated, they are: *Aspergillus niger, Aspergillus flavus*, *Penicillium expansum* and *Botrytis aclada*. These organisms were frequently encountered during isolations. Similar study was also conducted by Ibrahim (2005) and Joon *et al.* (2001) who indicated that *Aspergillus* and *Botrytis* species were most frequently encountered during isolations in many parts of the tropical and humid regions. This indicated that *Botrytis aclada* and *Aspergillus niger* were ubiquitous being encountered in all the three localities. Similarly, The disease incidence in the Study areas showed that *Aspergillus niger* had the highest frequency of occurrence of 96% in Woloji, 90% in Gulumba and 75% in Bama. Also, Gulumba had the highest frequency of occurrence of 75% *Botrytis aclada,* followed by 60% in Bama while least incidence of 30% was recorded in Woloji respectively. Meanwhile, *Penicillium expansum* had 45% frequency of occurrence in both Bama and Gulumba and not encountered in Woloji. Yohalem *et al.* (2003) also observed that *Aspergillus* and *Botrytis* species were most prevalent and ubiquitous because of their wide geographical distribution with devastating effects on onions and other vegetable crops in hot and humid regions.

The pathogenicity test revealed that *Aspergillus niger* was the most virulent when compared with the other test organisms because it had the highest mean rot diameter of 40.00 mm, followed by *Botrytis aclada* with mean rot diameter of 37.30 mm, *Aspergillus flavus* with mean not diameter of 28.40 mm and *Penicilium expansum* with the least mean rot diameter of 21.10 mm respectively. All the isolated organisms were pathogenic in causing neck rot diseases of onion. Joon et *al.* (2001) and Ibrahim (2005) also reported the same thing, that *Aspergillus niger* was the most virulent and prevalent in onion bulbs rot. Furthermore, *Botrytis aclada* and *Penicillium expansum* showed significant reduction in conidial growth of *A. flavus* and *A. niger* while treated with Neem leaf extract (*Azadiratcha indica* L.). In other related studies Suleiman and Falaiye (2013) reported that Neem leaf extract was effective in suppressing mycelial growth of *A. niger* causing biodeterioration in sweet potato.

The results of the treatment with different concentration of plant extracts revealed that Mahogany (*Khaya senegalensis* A Juss.) extract highly retarded the vegetative growth of the fungi responsible for the neck rot disease of onions because all the concentration levels (10, 20, 30 and 40)% showed least average mean values of (9.75) and (4.49) with LSD values of (1.03). The study further revealed the effectiveness of these three plant extracts in order of their LSD values as follows, Bitter leaf extract (3.28), Neem leaf extract (2.41) and Mahogany leaf extract (0.93). This indicated that Mahogany leaf extract was highly fungicidal than the other two plant extracts, similar observation was also made by Liman *et al. (*2010) who confirmed that Mahogany extract (*Khaya senegalensis* A Juss.) was highly effective on the control of Root knot disease of tomatoes caused by nematodes. It was observed that Bitter leaf (*Vernonia amygdalina* Del.) extract only retarded the vegetative growth of the fungi responsible for the neck rot disease of the onions at 30% concentration levels with the mean values of (22.17) and the LSD values of (3.67). This observation was different from the works of Suleiman and Ogundana (2010) who reported that aqueous solution of Bitter leaf extract (*Vernonia amygdalina* Del.) gave 100% inhibition of the vegetative growth of *Aspergillus niger*, *Cephalosporium* and *Trichoderma viridae* responsible for the deteriorations of cashew nut. These observed differences in the studies could be due to the extraction procedures of the plant extract, the concentration levels used and the duration of the experiment. Ijato (2011) made similar observation and reported that an aqueous extract of 80 % *V. Amygdalina* had high inhibitory effect against *Geotricum*, *Candida* and *Aspergillus niger* than 60 % aqueous concentration of the test plant extract while 30 % ethanolic extract of *V. Amygdalina* inhibited *Fusarium oxysporum* more than 20 % ethanolic extract.

Conclusion

The findings from this study revealed most of the spoilage of onions in the three Districts of Bama, Woloji and Gulumba are caused by these four isolated fungi, they are: *Aspergillus niger*, *A. flavus*, *Botrytis aclada* and *Penicilium expansum*. All were found to be highly pathogenic on Onion bulbs in storage. The study further indicated that the three Plant extracts had fungicidal properties with mahogany leave extract generally most effective than Bitter leaves and Neem leave extract in retarding vegetative growth of fungi in invitro with increase in the concentration of the extracts compared with the control. Plant materials are cheaper to obtain and not harmful to lives than the pesticides which are harmful and often costly. Famers are hereby recommended to use these measures in reducing neck rot diseases on their onions.

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