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Isolation and Management of Fungal Pathogens Associated with Neck Rot Disease of Onions (*Allium cepa* L.) in Maiduguri, Borno State

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Abstract A survey of some fungi associated with onion bulbs rot was carried out in Maiduguri, Borno State Nigeria. The aim of the study was to isolate and identify the fungal pathogens that cause the onion bulb rot. Onion samples were collected in three markets within Maiduguri, namely, Gamboru, Monday market and Baga markets. Four fungal pathogens were identified *as Aspergillus niger*, *A. Flavus*, *Botrytis aclada* and *Penicillium expansum*. Pathogenicity test conducted indicated that all the isolates were pathogenic on onions, thus causing rot. However, *A. niger* was the most virulent of the other isolates with highest mean rot diameter of 40.0 mm and *P. expansum* least virulent with only mean rot diameter of 21.00 mm. *A. niger* was prevalent in Gamboru market (96%), Monday market (90%) and Baga (75%), while least encountered was *P. expansum* with disease incidence of (45%) in Baga and Monday market respectively. Three plant extracts were tested on the organisms isolated. Of these plant extracts experimented. Mahogany leaves extract was highly effective against the organisms than the other plants extract and affordable compared to the pesticides which are poisonous and costly.

Keywords *Aspergillus niger, Aspergillus flavus, Botrytis aclada, Allium cepa, Penicillium expansum*

Introduction

Onion (*Allium cepa* L.) is one of the most important vegetable crop grown on a large scale in the world especially India and USSR for consumption and export [1]. Onion is classified under the Kingdom – *Plantae*, Class – *Angiosperm*, Order – *Asparagates*, Family – *Amaryllidiaceae* and Genus – *Allium* [2]. Hill and Waller (1988) [3] indicated that onions have been attacked by many diseases namely; downy mildew, purple blotch, blast and neck rot, whiterot, rust, smudge, leafspot, pinkrot and basal rot etc.

Raju and Naik (2007) [4] also indicated that onion are susceptible to many post harvest diseases such as soft rot, brown rot and smudge. They also reported that these diseases leads to substantial losses of the crops leading to unavailability of the crop in the markets.

White and Zelner (2008) [5] also indicated that onions are susceptible to number of diseases and disorders caused by fungi, bacteria and viruses, these diseases include Downy mildews, onion smut, onion smudge, white rot, neck rot, soft rot and Aster yellow.

Joon, *et al.* (2001) [6] indicated that major pathogens associated with basal and neck rot were: *Fusarium oxysporum*and *Aspergillus spp* or *Botrytis spp*respectively. Ibrahim (2005) [7] reported that most of the spoilage of onions in Borno State was caused by *Aspergillus niger*, through other diseases such as *Botrytis spp*also play very significant role. He further stated that onion cultivation is financially attractive and most importantly an essential component of the daily food intake in every home.

2. Materials and Methods

2.1. The samples for the study were collected in three different markets within Maiduguri metropolitan, thus, Gamboru market, Monday market and Baga market. The samples were obtained in the month of March – April (2015) being the season for the harvest.

2.2. Sample Collection

The infected onions were separated from the healthy ones and the disease incidence was determined. The samples were kept in separate polythene bags, labelled and taken to the laboratory for further investigation.

2.3. Estimation of Disease Incidence

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

2.4. Sterilization

The inoculating needle and the corkborer were sterilized by flaming using a spint lamp. The glass wares such as the petri dishes, the cornical flasks and test tubes were sterilized in a hot air oven at 160^{o}C for 2 hours and 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 (PaD.A)

Composition

Potato (Peeled/sliced)	200g
Dextrose	20g
Agar	15g
Distilled water	100ml

2.5.1. Preparation of Potato Dextrose Agar Medium

Irish potato tubes were peeled and sliced into the pieces and boiled in 500ml of distilled water for 15 minutes. The potato solution was decanted into a beaker and the slice discarded. In a separate beaker 500ml of distilled water was boiled while agar was slowly added and stirred with a glass rod to avoid lump formation and dextrose was dissolved in the potato infusion. The melted agar and the potato infusion were thoroughly mixed and made to volume of 1000ml by adding a distilled water. The medium prepared was transferred into a greshErlenmeyer flask and plugged with no absorbent cotton wool. This was 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 microbial growth. Then cooled and 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 distilled water three times. The rinsed diseased portion were cut into tiny pieces and blotted dry with sterile Whatman No. 1 filter paper. Thereafter, the onions were aseptically plated into 9 cm petri dishes containing the solidified PDA. Distinct colonies from the isolation plates were then subcultured to obtain the pure culture.

2.7. Preparation of Slides

A small portion of the fungal colony was scraped from the plate using an inoculating needle into the glass slide. 1-2 drops of laptophenol 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) [8-9].

2.9. Pathogenicity Test

Fire healthy onion bulbs were pierced with a sterilized 5mm cork borer to make a wound on each. Inoculums were introduced into the wounds using a sterile inoculating needle. The tissues were then replaced and covered with a masking tape and labelled and inoculated at 30 ^{o}C for 7 days. This was constantly observed and isolated and compared with the original using Koch's postulate. Inoculums were not introduced into the control but other treatment remained equal.

2.10. Preparation of Leave Extract

Three different plant leaves, thus, African Mahogany (*Khaya senegalensis*), Neem (*Azadratchaindica)* and Bitterleaf (*Vernonia amygdalina)* were thoroughly washed under a running tap water, air dried separately and micronized using pestle and mortar into fine powder. Cold water extraction was then obtained by adding 10, 20, 30 and 40g 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 cheese cloth to obtain 10, 20, 30 and 40 % crude aqueous extract [10].

2.11. Control Using Leaf Extract

Ten milliliters of an appropriate dilution of each plant extracts poured into 9cm petri dishes with solidified potato dextrose agar and drained off. Then a 3 day old colony was cut using a sterile 5mm 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 and observed constantly.

2.12. Experimental Design and Statistical Analysis

A completely randomized design was used as described by Gomez and Gomez (1984) [11]. 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 neck rot disease of onions in Maiduguri, four fungal pathogens have been identified as *Aspergillus niger, A. Flavus, Botrytis aclada and Penicillium expansum* (Table 1)

Table 1: The Results of the Fungal pathogens in Maiduguri, in the 3 markets.

Organism	Gamboru market	Monday market	Baga market
Aspergillus niger	✓	✓	✓
Aspergillus flavus	✓	-	✓
Botrytis aclada	✓	✓	✓
Penicillium expansum	-	✓	✓

KEY ✓ = Isolated - = Not Isolated

3.2. Estimation of Disease Incidence

*Aspergillus niger*had the highest incidence of 96% in Gamboru market, followed by 90% in Baga market. And Monday market had 75% incidence. Furthermore, Baga market had the highest incidence of 60% *A. flavus* followed by 45% in Gamboru market while not encountered in Monday market. Also, *Botrytis aclada* had highest incidence of 75% in Baga market and 60% in Monday market. Least encountered was recorded in Gamboru market with 30% incidence respectively. *Penicilium expansum* had 45% incidence in Monday market and Baga not encountered in Gamboru market (Table 2)

Table 2: The Disease Incidence in Maiduguri

Organism	Gamboru market	Monday market	Baga market
Aspergillus niger	75	96	70
Aspergillus flavus	00	45	60
Botrytis aclada	60	30	75
Penicillium expansum	45	00	45

3.3. Pathogenicity Test

The pathogenicity test revealed that all the organisms isolated were pathogenic on onion bulbs, however, *Aspergillus niger*was the most virulent of all the other organisms isolated because it had the mean rot diameter of 40.00mm

folled by *Botrytis aclada*(37.30mm). *A. Flavus*(28.40mm) and the least pathogenic was the *Peniciliumexpansum*with mean rot diameter of (21.00mm) (Table 3).

Table 3: The Results of Pathogenicy Test of Isolated Fungal Pathogens Incubated for 7 days

Organisms	Rot diameter (mm)
Aspergillus niger	40.00
Aspergillus flavus	28.40
Botrytis aclada	37.30
Peniciliumexpansum	21.00

3.4. Effects of Leave Extracts on the Isolated Organisms

Higher concentrations of 30-40% of the extract control the vegetative growth of the organisms. Mahogany leaf extract was effective in controlling the vegetative growth of the organisms at all the concentration levels applied as shown below (Table 4).

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

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

Key ** = Highly Significant at (p=0.001) cont = control

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