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Identification of Fungal Pathogens of Postharvest Rot of Sweet Melon Fruit (*Cucumis melo* L.) In Jimeta, Yola-North Local Government Area of Adamawa State

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Abstract- ***Sweet melon is an important fruit crop consumed and recognized globally. However, its production and storage have been devastated globally over the years due to pathogenic infections, the most serious one is the postharvest spoilage caused mainly by microorganisms such as fungi. Identification of fungal pathogens of postharvest rot of sweet melon fruit (Cucumis melo) was conducted in the laboratory of Plant Science Department of Modibbo Adama University of Technology, Yola. The experimental design used was Completely Randomized Design (CRD), each treatment was replicated three times. Data collected was analyzed using ANOVA as statistical tool, and means were separated using the Least Significant Difference (LSD). The survey conducted, indicated that Gwari market has the percentage incidence of rot (26%) and mean radial diameter of rot (23.4mm), Jimeta modern market had percentage incidence of rot (30%) and mean diameter of rot (37.4mm) and Jimeta shopping complex with the highest percentage incidence of rot (36%) and mean diameter of rot (50.1mm). The fungi that were isolated from rotten sweet melon fruits include: Aspergillus niger, Pseudallescheria boydii, and Neosartorya pseudofischeri. The Pathogenicity test results indicated that all the isolated fungi were pathogenic on sweet melon fruits, however, A. niger was the most virulent of the other test organisms because it had the highest mean rot diameter of (282mm) followed by Pseudallescheria boydii with (266mm) and Neosartorya pseudofischeri had (236mm). It is recommended that further research be carried out on the effect of infection on the nutritional value of sweet melon fruits.***

Keywords: ***Identification, Fungal, Pathogens, Postharvest Rot Sweet-melon***

I. INTRODUCTION

Sweet melon is a very important fruit crop because it is low in calories and fats (Agricultural Marketing Service, 2004). The fruit is rich in numerous health promoting compounds, vitamins, and minerals that are absolutely necessary for optimum health (Janick and Paris, 2006). Honeydew melon fruit is an excellent source of vitamin A one of the highest among cucurbita fruits (Ceponis, Wells and Cappellini, 2005). Vitamin A is essential for healthy vision, it is also required for maintaining healthy mucus membranes and skin (Chuku and Emelike, 2013). Consumption of natural fruits rich in vitamin A has been known to help protect people from lung and oral cavity cancers (Wells and Ceponis, 2006). Honeydew melon fruit is also rich in antioxidant flavonoids such as beta-carotene, lutein, zea-xanthin and cryptoxanthin. These antioxidants offer protection against colon, prostate, breast, endometrial, lung, and pancreatic cancers (Irey and Stall, 2001).

In a hungry and increasingly competitive world, reducing postharvest food losses is a major concern to plant pathologists (Olesen, Nacey, Wiltshire and O'Brien, 2004). For highly perishable commodities, such as honeydew melons, tomatoes, water melons, oranges guava, etc. as much as 30 percent of the harvested crop may be lost to postharvest diseases before it reaches the consumer (Stepansky, Kovalski, and Perl-Treves, 2006). Investments made to save food after harvests are usually less costly for the grower and the consumer and less harmful to the environment than efforts to increase production (Fallik, 2004). Even a partial reduction in postharvest losses can significantly reduce the overall cost of production and lessen our dependence on marginal land and other scarce resources (Potter, 1992).

Many factors contribute to postharvest losses of fresh sweet melon fruits (Olesen, Nacey, Wiltshire and O'Brien, 2004). These include environmental conditions such as heat or drought, mechanical damage during harvesting and handling, improper postharvest sanitation, and environmental control (Ben-Yehoshua, 2003; Sharma and Sharma, 2006). Efforts to control these factors are often very successful in reducing the incidence of disease (Bailey and Bailey, 2006). For example, reducing mechanical damage during grading and packing

greatly decreases the likelihood of postharvest disease because many disease-causing organisms (pathogens) such as fungi and bacteria enter through wounds (Ryall and Lipton, 2009). Honeydew or sweet melons, as it is often called in Nigeria surfers from various effects of postharvest spoilage due to many factors that include environmental and the effect of postharvest pathogens (Mapanda, *et al.,* 2005).

Post-harvest losses in Honeydew melon fruits are often due to fungal rots (Bokshi, Morris, McConchie, and Deverall, 2006). These rots occur on the external surface of the fruit and gradually progress inwards into the flesh (Bokshi *et al.,* 2006). Fungal pathogens of major concern are species of *Alternaria, Penicillium, Cladosporium, Rhizopus, Fusarium and Aspergilus. Alternaria and Cladosporium.* Hence the aim of this work is to identify the fungi responsible for the postharvest rot of sweet melon fruit (*Cucumis melo L.*) in Jimeta, Yola-North Local Government Area of Adamawa State.

II. MATERIALS AND METHODS

Area of Study

Survey was randomly carried out in Gwari Market, Jimeta Modern Market and Jimeta Shopping Complex. Isolation, identification and control trials were conducted in the Department of Plant Science Laboratory of Modibbo Adama University of Technology Yola in Adamawa State between the months of July and August, 2016. Adamawa state is located at the north eastern part of Nigeria and lies between latitude 7^0 and 11^0 north of the equator and between longitude 11^0 and 14^0 east of the Greenwich meridian (Adebayo, 1999).

Adebayo (1999) gave a detailed description of the climatic condition of the region. The area has tropical climate marked by dry and rainy seasons, the rainy season commences around May and ends in the middle or late October while the dry season starts at October or November and lasts to April. The main annual rain fall ranges from 700 mm in the north western part to 1600 mm in the southern part of the state. Maximum temperature is about 40^0C around April while minimum temperature could be as low as 18.3^0C between December and early January. Relative humidity in the area is about 26% in the month of January while February has the lowest value of 16%, the month of July and august usually have the peak with relative humidity of about 80%.

Sources of Samples

Samples of honeydew or sweet melon fruits showing rot symptoms were collected from Jimeta Modern Market, Jimeta Shopping Complex and Gwari Markets all within Jimeta in sterile polythene bags, straight to the laboratory of Plant Sciences Department of Modibbo Adama University of Technology Yola for isolation and identification between the months of February to April 2016. Jimeta is an area within the capital city of Adamawa State (Figure 1), with major and minor markets where different types of fruits (including sweet melon fruits) are sold. There is frequent postharvest spoilage of fruits and vegetables this is the main reason for the choice of the study area. The sample size is 50 honeydew melon fruits from each market, making a total of 150 honeydew melon fruits from all the markets.

Determination of Sweet Melon Rot Incidence and Severity in the Market

The incidence of the honeydew melon fruit rot in each market was determined by counting the infected honeydew melon fruit from the samples collected from each market. The formula is given by:

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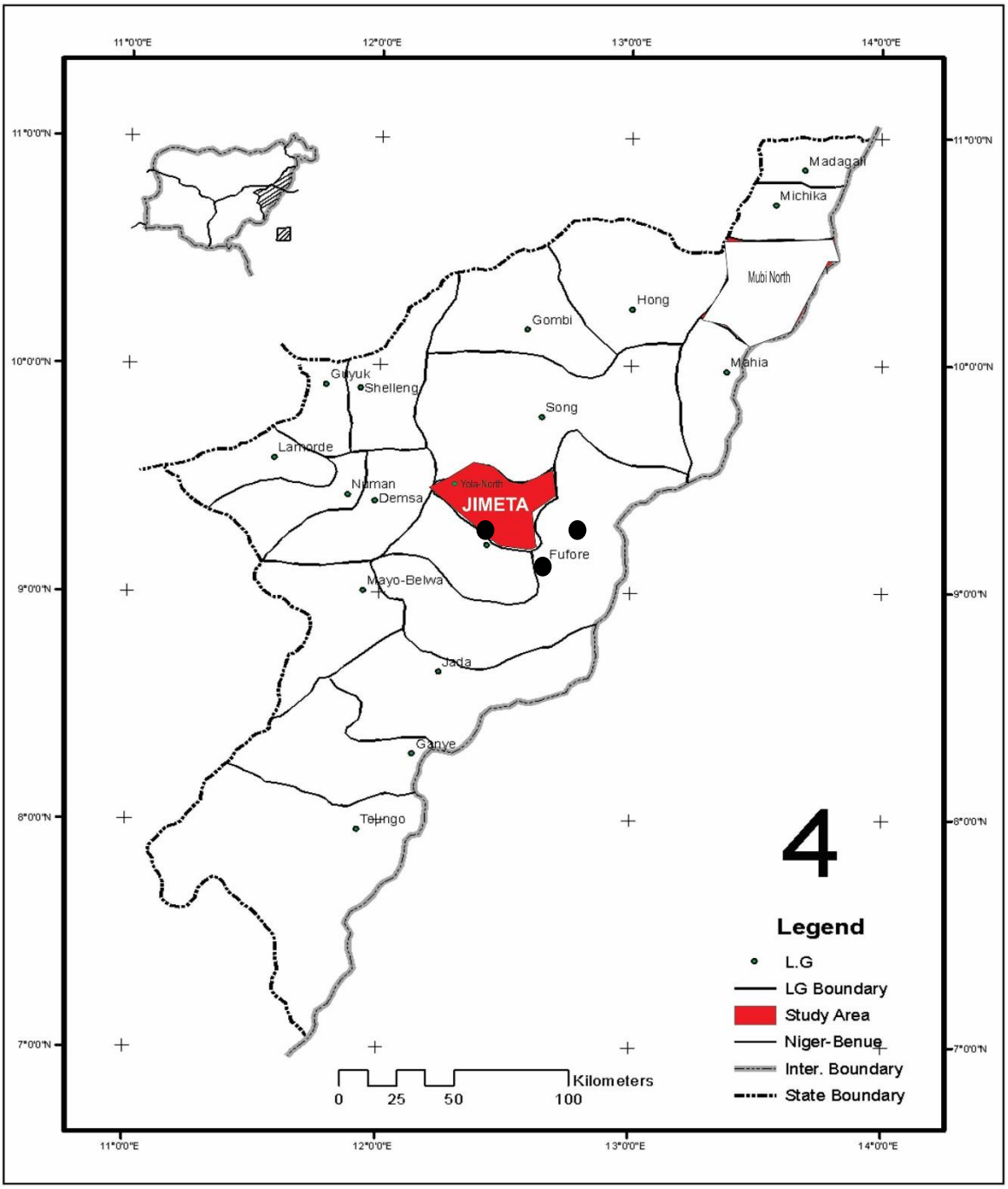

Figure 1: Map of Adamawa State (Showing The Study Area)
Source: Department of Urban and Regional Planning (MAUTECH)

$$\%\text{Disease Incidence} = \frac{\text{Number of infected Honeydew melon fruits}}{\text{Total number of Honeydew melon fruit collected}} \text{ X } 100$$

Severity of the honeydew melon fruit was assessed by ruler measurement of size of rot and recorded in millimeters.

Preparation of Culturing Medium

Potato dextrose agar (PDA) was used for culturing of the isolates, and was prepared according to the method of Suleiman and Michael (2013).

Isolation and Storage of Fungi

Under aseptic conditions the diseased sample of sweet melon fruit showing rot was cut into approximately 5 mm with a neat sterile scalpel. Pieces were picked with flamed, and then cooled pair of forceps. The pieces were immersed into 0.1% sodium hypochlorite contains in a sterile 9 cm diameter Petri- dish for surface sterilization for 30

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seconds. The sterilized pieces were rinsed in three changes of sterile distilled water and then blot dried between sterile filter papers. With a flamed and cooled pair of forceps, a sterilized piece of sweet melon fruit was then plated aseptically on 9 cm diameter Petri-dish containing sterile solidified Potato Dextrose Agar (PDA) and incubated at room temperature of 30±3^{0}C within the months of July and August, for 3-4 days (i.e. immediately when new colonies begin to grow) before sub- culturing on fresh sterilize PDA using the method of Pitt and Hocky, (1997) and Timko *et al*. (2008). Pure colonies were photographed and photomicrograph under microscope (x40) was also taken. Pure isolates of fungal species obtained were stored on solidified sterile PDA in McCartney bottles. These were appropriately labeled according to organism and location. The slant was initially corked loosely to the content fungus to grow. They were tightly corked and stored at a temperature range of 0–4^0 C in a refrigerator to serve as stock culture until when needed.

Preparations of Slides and Identification of Fungi

Microscopic examination was carried out to observe the structure and characteristics of the fungal isolates. A sterile needle was used to pick a little portion of the hyphae containing spores and placed on a sterile glass slide stained with Lacto phenol cotton blue and examined under the photographic microscope using the method of Fawole and Oso (1995). Micrograph of the isolates showing (conidia, sporangia etc.) were taken. The morphological and cultural characteristics observed were compared with structures in the identification guides by Hunter and Barnet (1998).

Frequency of Occurrence

The prevalence of the isolated fungi of sweet melon fruit rot was calculated and expressed as percentage frequency as follows:

$$\% \text{ Frequency} = \frac{\text{Number of isolated organisms X 100}}{\text{Total number of isolated organisms X 1}}$$

Pathogenicity Test

It was determined according to the method of Chukwuka *et al.* (2010).

Data Analysis

All the data obtained were analyzed using analysis of variance (ANOVA) to test for significance using statistical tool for applied sciences (SAS) version 8.3 and the means that were significant were separated using the least significant difference (LSD) at 5% probability level (Schaffer *et al.,* 2010).

III. RESULT

Incidence and Severity of Sweet Melon Rot in Jimeta

The result on Table 1 shows that soft rot of sweet melon was prevalent in all the three markets surveyed. The percentage incidence of the rot varied significantly among the markets P=0.05. The percentage incidence of rot in Jimeta Shopping Complex was the highest 36% followed by Jimeta Modern Market 30% and Gwari market had the lowest 26%. The Table also shows the extent of damage (i.e. severity) on infected samples of sweet melon fruits rot in Jimeta. There was a significant difference in the severity of rot found in the three markets at P=0.05. Jimeta Shopping Complex having the largest rot with lesion size of 50.1 mm, followed by Jimeta Modern Market with moderate severity of infected samples 37.4 mm and Gwari Market also has lowest severity of infected samples 23.4 mm diameter lesions.

Identification of Fungal Isolates

The isolated fungi from Sweet melon fruits were identified as *Aspergillus niger, Pseudallescheria boydii* and *Neosartorya pseudofischeri* (Plates 1-3). These were identified based on their colonial and morphological characteristics. The result on Figure 2 shows the radial growth expansion rate of the three isolates for seven days with *Aspergillus niger* having the highest growth of 90 mm followed by *Pseudallescheria boydii* with78 mm and *Neosartorya pseudofischeri* has the lowest radial growth of 71mm this figure displays bars with standard error of 5% value.

Table 1: Percentage Incidence and Severity of Sweet Melon Rot in Jimeta, Adamawa State, Nigeria

Markets	Incidence of Rot (%)	Rot diameter (mm)
Gwari Market	26	23.4
Jimeta Modern Market	30	37.4
Jimeta Shopping Complex	36	50.1
LSD (0.05)	3.37	3.31

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Key
LSD: Least Significant Difference

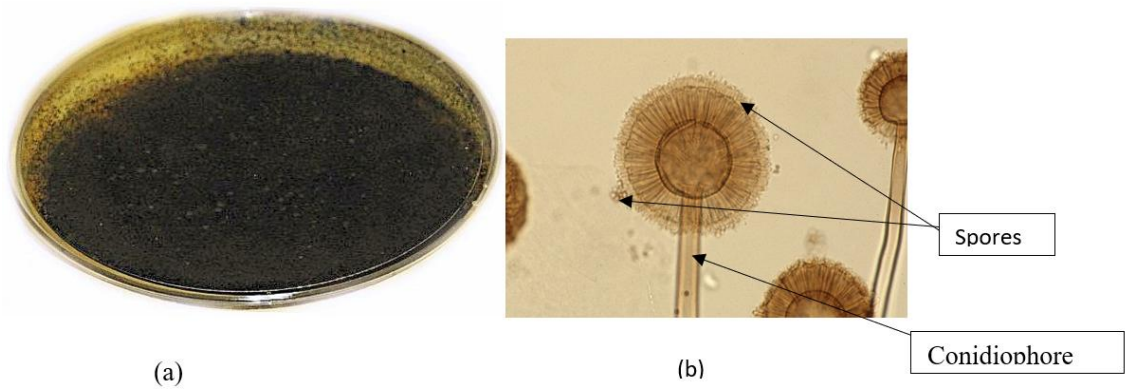

Plate I (a): Seven 7-day old culture of *Aspergillus niger* (×1)

Plate (b): Micrograph of *A. niger* with spores from conidiophore and thick-walled and hyaline conidiophore (×40)

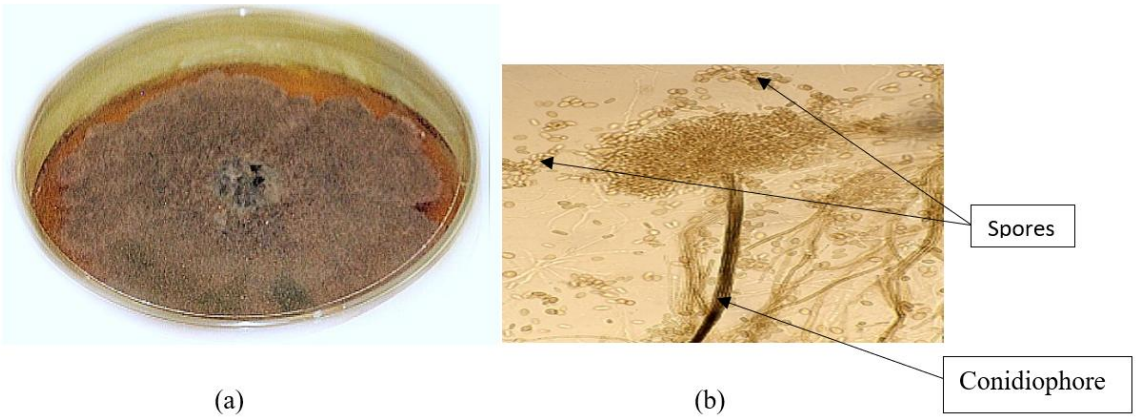

Plate II (a): Seven 7-day old culture of *Pseudallescheria boydii* (x 1)

Plate (b): Micrograph of *Pseudallescheria boydii* showing spores and conidiophore (x 40)

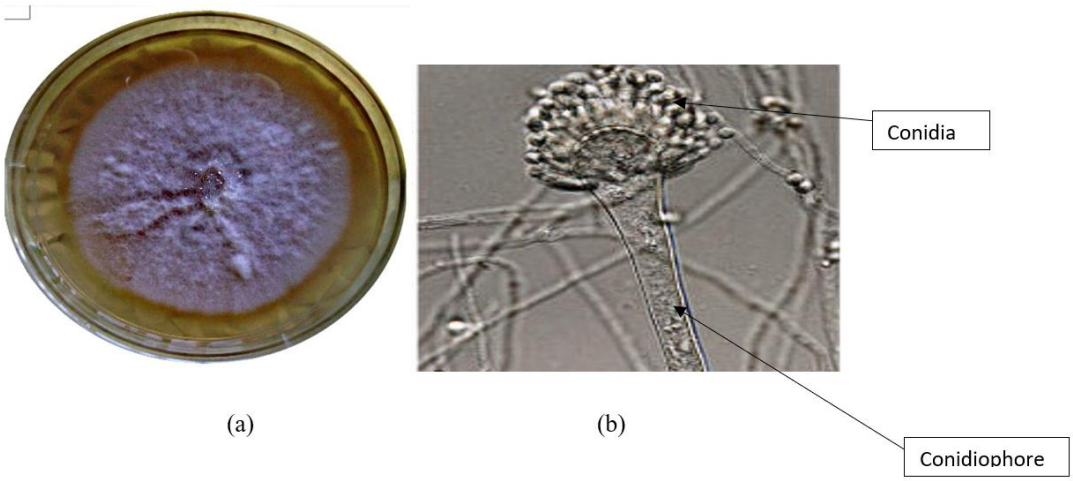

Plate III (a): Seven 7-day old culture of *Neosartorya pseudofischeri* (x 1)

Plate (b): Micrograph of *Neosartorya pseudofischeri* showing conidiophore (x40)

Table 2 depicts the occurrence of fungi found associated with marketed sweet melon fruit in three different markets in Jimeta. All the samples investigated from the three markets harbor *Aspergillus niger*, *Pseudallescheria boydii* and *Neosartorya pseudofischeri* except Jimeta modern

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market which does not have *Pseudallescheria boydii*. *Aspergillus niger* had the highest frequency of occurrence in all the three markets with mean frequency of 50.01%, while *Neosartorya pseudofischeri* had the lowest frequency of occurrence 22.91 (Table 3).

Pathogenicity of Fungal Isolates on Sweet Melon Fruits

The result of test on virulence of isolates revealed that all the isolates were pathogens, since there was a very highly significant difference between the means of the infected fruits and the non-infected fruits (control) that showed no rot at all at P=0.05 (Table 4). There was a significant variation in virulence among the pathogens with *Aspergillus niger* having the highest virulence producing rot lesion of 282 mm followed by *Pseudallescheria boydii* 266 mm and then *Neosartorya pseudofischeri* 236 mm.

Table 2: Occurrence of Fungal Pathogens of Sweet melon Fruit Rot in three markets in Jimeta, Adamawa State, Nigeria

Fungal isolates	Presence of isolates in each market		
	GM	JMM	JSC
Aspergillus niger	+	+	+
Pseudallescheria boydii	+	_	+
Neosartorya pseudofischeri	+	+	+

Key:
GM: Gwari Market
JMM: Jimeta Modern Market
JSC: Jimeta Shopping Complex
\+ Presence
_ Abscent

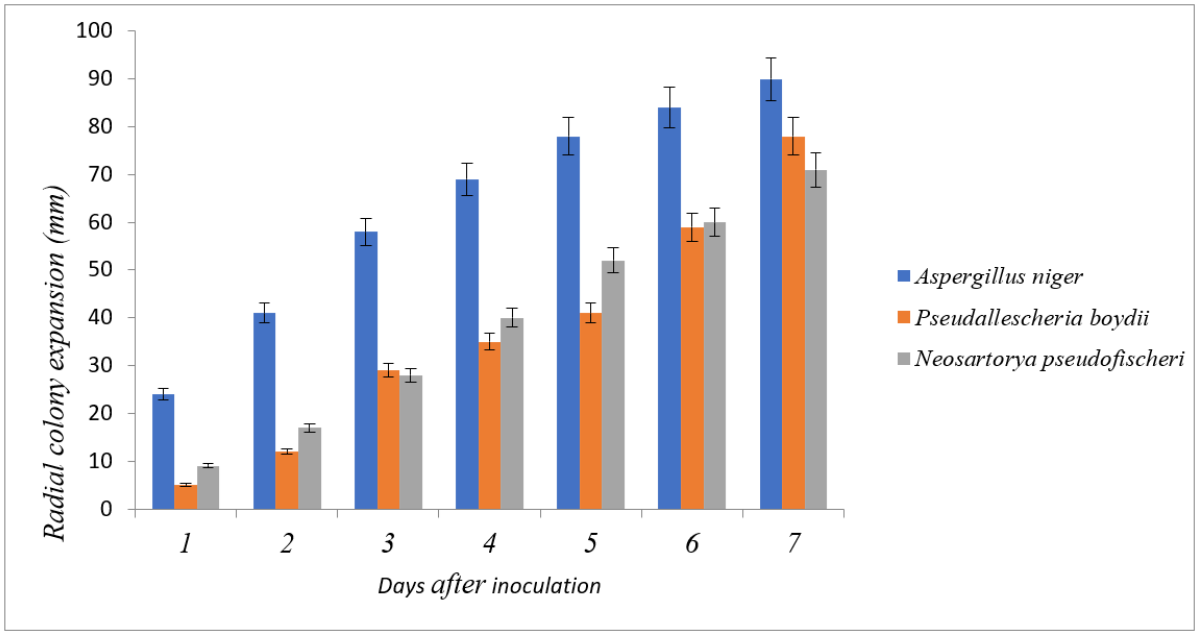

Figure 2: Radial Growth of the Three Fungal Isolates in the Petri Dishes for Seven (7) Days

Table 3: Frequency of Fungal Pathogens of Sweet melon Fruit Rot in Jimeta (%)

Fungal isolates	Frequency of isolates per market (%)			
	GM	JMM	JSC	Mean
Aspergillus niger	44.50	51.67	54.24	50.01

Pseudallescheria boydii	33.37	11.50	33.38	27.08
Neosartorya pseudofischeri	22.23	36.83	12.38	22.91
Total	100	100	100	100
LSD (0.05)	0.33	0.34	0.30	0.43

Key
GM: Gwari Market
JMM: Jimeta Modern Market
JSC: Jimeta Shopping Complex
LSD: Least Significant Difference

Table 4: Virulence of the Isolates on Fresh Sweet melon Fruit (mm)

Fungal Isolates	Lesion size (mm)
Aspergillus niger	282
Pseudallescheria boydii	266
Neosartorya pseudofischeri	236
Control	0.00
LSD (0.05)	2.88

Key
LSD: Least Significant Difference

IV. DISCUSSION

The survey of postharvest rot of sweet melon fruits in Jimeta showed that soft rot of sweet melon was prevalent in all the three markets surveyed. The finding of this study revealed that Jimeta Shopping Complex had higher incidence of sweet melon fruit rot of 36%, followed by Jimeta Modern Market with 30%, and Gwari Market 26%. These incidences are moderate when compared with 5-point scale by Ratanachadcheil *et al.* (2010). This is in agreement with the report made by Zakawa (2015) that fruit rot occurred in all location with average mango fruit rot incidence found to be common in Jimeta Modern Market, Jimeta Shopping Complex and Jimeta Old Market. Also, the finding made by Anjili, Channya and Chimbekujwo, (2016) similarly reported that Jimeta Shopping Complex had the highest frequency of rot, followed by Jimeta Old Market and Jimeta Ultra-Modern Market respectively. The abundance of water in the tissue of soft sweet melon fruit may account for rot recorded in this study as reported earlier by Defosent (2012) that soft or pulpy fruits undergo a soft rot because of the abundance of water in their tissue.

The severity (extent of damage) of rot on the sampled sweet melon fruits in Jimeta showed that sampled fruits from Jimeta shopping complex having the highest rot severity 50.1mm, followed by Jimeta modern market with moderate severity of fruit's rot 37.4mm and Gwari market with least severity of rot 23.4mm. The results of incidence and severity of rot in the three markets surveyed agreed with earlier reports made by Mafindi (2010), Gadgile and Chavan (2010) that fruits rot are common in northeastern Nigeria markets.

The high moisture content and soft texture of sweet melon fruits, make them susceptible to mechanical injury as recorded in this study, which can occur at every stage from production to retail marketing because of poor harvesting practices. Also unsuitable field or marketing containers and crates, which may have splintered wood, sharp edges, poor nailing or stapling, over packing or under packing of field or marketing containers. Careless handling, such as dropping or throwing or walking on produce and packed containers during the process of grading, transport or marketing (FAO, 2013).

Also, this study showed that different fungi were associated with postharvest rot diseases of sweet melon fruit in the study area (Yola-North Local Government, Adamawa State). The fungi isolated include; *Aspergillus niger*, *Pseudallescheria boydii* and *Neosartorya pseudofischeri* this finding is in line with reports made by Anjorin, Adebayo and Hussaini (2013) that eight different fungi were found associated with fruit rot; *Aspergillus carbonarius,*

Aspergillus tereus, Aspergillus flavus, Aspergillus candidus, Pseudallescheria boydii, Aspergillus niger, Candida tropicalis and Aspergillus glaucus. Likewise, the finding of variety of different fungi associated with postharvest rot also agreed with the reports made by Gurjar *et al.,* (2012) and Kiama *et al.,* (2015) that reported recovered variety of fungal isolates from rotten sweet melon fruits namely, *Trichoderma viride, Trichoderma spirale, Neosartorya pseudofischeri, Neosartorya aureola, Aspergillus flavus, Aspergillus terreus, Pseudallescheria boydii and Trichoderma longibrachiatum.* This is also similar to finding made by Amadi *et al.,* (2014) who reported a total of seven (7) isolated fungi from Guava fruit spoilage namely *Pseudallescheria boydii, Fusarium oxysporum, Neosartorya pseudofischeri, Rhizopus stolonifer, Aspergillus niger, A. fumigatus and A. parasiticus. F. oxysporum*.

The fastest growing among the three isolates was *Aspergillus niger,* The reason for this might be connected to some factors such as type of media used which is potato dextrose agar, which was expressed by Wina *et al.,* (2016) as best type of media for the culturing of these fungi. Also, the temperature of the study area, which is 30±2 ^{0}C in Yola at the month of July, this suggest that this classes of fungi are mesophylls growing best at the temperature range of 20-45^{0}C. Other reasons for the radial expansion of the three isolates may be as a result of the pH of the culture media which coincided with optimum growth pH of the isolates, light intensity on the growth medium, moisture etc. This findings agrees with the reports of Chuku and Emelike (2013) and Zongo *et al.,* (2011) that *Pseudallescheria boydii* and Scedosporium apiospermum moderately rapid *growing* colony at temperature of 25^{0}C, which matures in about a week. Likewise, this finding agrees with conclusion made by Reeth and Senthilkumar (2014) that most popular and *fastest growing* fungi include *Pseudallescheria*, Sporothrix, *Aspergillus, Neosartorya pseudofischeri*.

Result of pathogenicity test of the isolates showed that all the three fungi were pathogenic to the sweet melon fruit used for this study, although the degree of pathogenicity varied. They were not only able to grow on the fruits but also were able to induce some level of fruit rot indicating their virulence. Growth was not evident within the first 24hours after inoculation in all the isolates. Among the isolates, *Neosartorya pseudofischeri* exhibit the least level of virulence as compared to the other two (2) isolates. This is in line with earlier report by Joseph and Aworii (1992) and Gadgile and Chavan (2010) who reported the differences in the pathogenicity of fungal isolates from sweet melon fruits might be due to their ability to overcome the natural defense mechanism of the fruits or their ability to induce resistance in the fruits when infected. *Aspergillus niger* was rated as having moderate pathogenic effect in the fruits. This agrees with the result obtained by Srivastava *et al.* (2007) who found *Aspergillus niger* to be highly virulent in fruits and also by Gadgile and Chavan (2010), who reported that severity of the fungus was at its peak at 35^{0}C and 100% relative humidity when inoculated. *Pseudallescheria boydii* was rated as having high pathogenic effect in the fruits. Growth and rot cover of 266 mm of the sweet melon fruit surfaces.

V. CONCLUSION

There is high incidence and severity of sweet melon fruit rot in Jimeta caused by pathogenic fungi. Toxigenic fungi that include *Neosartorya pseudofischeri*, *Pseudallescheria boydii* and *Aspergillus niger* are found associated with sweet melon fruit rot. *Aspergillus niger* is a serious threat to sweet melon fruit storage because of its high frequency of occurrence and virulence level.

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