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Importance and Pathogen Spectrum of Crown Rot of Banana in Jimma Town, Southwestern Ethiopia

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Abstract
The present study was conducted to determine the prevalence of crown rot of banana in Jimma town and to identify the primary fungal pathogens associated with crown rot of banana. A total of four markets at Jimma town were surveyed. From each market ten banana retailers were considered for sample collection. Sample collected from this market were used to determine major pathogens associated with the disease. The survey revealed that, crown rot was found widely distributed on banana fruit in all sampled area, with 100% prevalence. A total of six fungi comprising five genera namely Colletotrichum musae, Aspergillus flavus, Aspergillus niger, Fusarium spp, Penicillium sp. and Rhizopus spp were found associated with the evaluated banana fruits. Among which Colletotrichum musae (46.86%) was found to be the most prevalent. The present study reviled that crown rot is the most prevalent and major challenging disease of banana caused by several fungi, which threatened the utilization of banana throughout the town. Thus, extensive research needs to be conducted across different districts to come with tangible recommendation to develop appropriate management tool to reduce the losses.
Key words: Banana, Crown rot, Postharvest, Prevalence

1. Introduction

Banana (*Musa* spp.) is one of the most popular fruits grown throughout the tropics and subtropics worldwide (Ewane *et al*., 2012). It is consumed both as an energy yielding food and as a dessert. Banana is a very important staple commodity in many developing countries and is the main fruit in international trade which ranks first in terms of volume exported and second after citrus fruit in terms of monetary value (Anthony *et al*., 2004; Ewane *et al*., 2012). However, because of their high moisture and the nutrient reserve, banana fruits are highly susceptible to different pathogenic fungi during the period between harvest and consumption. The fruit is the host of a large number of pathogens among which fungi could be major agents of fruit rot after harvest in the world. Several different fungal diseases reduce the quality and postharvest shelf life of this fruit crop (Win *et al*., 2007). The postharvest diseases of banana include crown rot, anthracnose, cigar-end rot, ripe rot, stem-end rot and black end. Of these crown rot is by far the most economically important disease of banana (Krauss *et al*., 2000; Lassois *et al*., 2011).

It is characteristically a disease complex caused by several fungi, sometimes in association with other micro-organisms such as bacteria (Krauss *et al*., 2000; Lassois *et al*., 2011). In some countries, losses as high as 86% have been observed in bananas without chemical treatment and during the rainy season (Alvindia *et al*., 2000). In Ethiopia also, crown rot is one of the most important and widely distributed diseases of ripe and ripening banana which threatened the production and utilization of banana in different parts of the country (Yesuf *et al*., 2009). Most of the commercial varieties of banana are susceptible to crown rot; the disease can cause considerable postharvest losses of the fruit and could, therefore, be considered as the great threat to local and export market banana.

In spite of the yield losses caused by postharvest diseases, sufficient attention has not been given to research on this group of diseases in Ethiopia (Yesuf *et al*., 2009). Hence, this study was conducted to assess the prevalence of crown rot of banana and to identify the primary fungal pathogens associated with crown rot disease of banana.

2. Materials and Methods

2.1. Filed Survey

2.1.1. Sampling and sample unit

A total of four markets in Jimma town, namely Bosa Kito, Hermata Markato, Hermata Mentina and Qochi Mendera were surveyed for crown rot disease during 2013 cropping season. From each market ten banana retailers were considered for sample collection, and samples of about 5 fruits per retailer in one hand were randomly taken for disease diagnosis in the laboratory. Fruits were stored at room temperature and inspected daily for symptom appearance, and then fungi were isolated and maintained in the laboratory for further analyses.

2.1.2. Determining prevalence of crown rot of banana fruits

Collected banana fruit samples from the four markets were kept in the Plant pathology Laboratory of Jimma University College of Agriculture and Veterinary Medicine and examined at 48 hours interval for incidence of crown rot and prevalence of the disease in the samples was also calculated. Disease incidence was determined as the percent of infected fruits based on the number of fruits showing symptoms of crown rot in each sample.

2.2. Laboratory Experiments

The laboratory experiments for determination of fungal pathogens associated with crown rot disease of banana were carried out in the Plant Pathology laboratory of Jimma University College of Agriculture and Veterinary Medicine in 2013 main cropping season.

2.2.1. Sources of Disease Tissues

Crown rot infected banana hands were collected from four selected markets (Bosa Kito, Hermata Markato, Hermata Mentina and Qochi Mendera) of Jimma town. Hands showing severe crown rot disease were selected and used for the work.

2.2.2. Isolation of fungi

Isolation of fungal pathogen associated with crown rot of banana was made from the advancing margins of the disease on the crown and the fruits. Diseased hands were carefully washed under running tap water and diseased tissues were aseptically removed from advancing edges of the rot. Tissues were then cut into smaller pieces with a sterile scalpel and surface sterilized with 0.5% of Sodium hypochlorite (NaOCl) solution for 5 minutes. These were then rinsed with three changes of sterile distilled water to remove traces of the bleaching agent used. Resulting tissues were evenly spread out in a plate and allowed to dry. Pieces of the dried tissues were aseptically removed and placed gently on a poured PDA plate. Four point plating was done. After incubation for one week at 25 ^{o}C the developing fungi were sub cultured and pure cultures of fungi were maintained further use.

2.2.3. Identification of fungi

Pure cultures of individual fungal isolates which are associated with crown rot of banana were critically examined and identified. Fungi were identified based on gross colony morphology and microscopic characters. Colony identification was based on colony characteristics such as color and texture of mycelia and type of pigmentation. Microscopic characteristics of spores such as shape and color were depended on in identifying the fungal isolates based on descriptions of Marin *et al*. (1996) and Holliday (1995).

2.4. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS V 9.0 software package. The data are the mean of triplicate experiments.

3. Results and Discussion

3.1. Prevalence of Banana Crown Rot

Three cultivars of banana, namely Arbaminch, Mizan and Local were recognized in different places of the sample collection areas. Arbaminch was originally obtained from Arbaminch and it was found abundantly in most surveyed markets. The source of mizan banana is from Mizan tepi. The local cultivar is from Jimma zone and its surrounding area. The retailers or traders transported and stored banana fruits in basket wooden boxes covered with paper to protect the banana from direct contact with sunlight, dirt and rainfall, thereby prolonging storage life of the fruit. However, such environmental condition will create good condition for the development of postharvest pathogen. During the field survey, crown rot was found widely distributed on banana fruit in all sampled locality, with 100% prevalence (Table 1). However, there was variation in levels of disease development due to pathogen associated with it and the handling mechanism.

3.2. Fungal Pathogen Associated with Crown Rot of Banana

A total of six fungi comprising five genera namely *Colletotrichum musae Aspergillus flavus, Aspergillus niger, Fusarium spp Penicillium* sp. *and Rhizopus spp* were isolated from banana fruit samples collected from four different markets at Jimma town (Table 2). The fungal colony and the morphology of the isolated fungi are demonstrated in Table 3 and Figure 1. Among the isolated fungi *Colletotrichum musae* was the most prevalent followed by *Aspergillus flavous* and *Aspergillus niger*, with average frequency of occurrence of 46.86%, 14.75% and 13.85%, respectively. On the other hand *Rhizopes* sp. was recorded as the lowest frequent pathogen in the surveyed area.

In the present study, *Colletotrichum musae* recorded the highest occurrence in all the four study areas with frequency of occurrence range from 44.19% at Hermata Merkato to 52.78% at Bosa kito. Such similar reports have been made by Finlay and Brown (1993), who reported that *C. musae* was the most aggressive species that caused extensive rotting of banana crowns when inoculated artificially. It is also reported that *C. musae* is a primary pathogen of banana crown rot and could initiate infection from low initial inoculum levels (Finlay and Brown, 1993; Krauss, 1996). Krauss (1996) also reported that banana crown rot and anthracnose are both caused, primarily by *C. musae* and both disorders cause symptoms during ripening that the fungus successfully penetrates a wound.

Present results revealed that saprophytic fungi viz., *A. flavus* and *A. niger* were predominant among the fungi isolated. *Aspergillus flavus* was mostly isolated from Hermata Merkato (20.93%) whiles *Aspergillus niger* was mostly isolated from Bosa Kito (19.44%) districts. Similarly, Anthony *et al.* (2004) states that *Aspergillus* spp. are one of the most commonly isolated microorganisms in banana crown rot. Some *Aspergillus* spp. are known to produce aflatoxins a group of highly toxic, mutagenic and carcinogenic polyketide compounds on several plant produce (Krogh, 1992). *Fusarium* spp recorded the highest frequency 15% at Qochi Mendera next to *Colletotrichum musae.* This in line with Finlay and Brown (1993) reported that different *Fusarium* spp. have been shown as a primary cause of banana crown rot in many countries.

Penicillium spp were recorded on 75% of the surveyed markets. It was reported that, *Penicillium* spp have been associated only occasionally with crown rot disease (Johanson and Blazquez, 1992) although they are very important postharvest pathogens in other crops, such as oranges and apples. Agrios (2005) indicated that *Penicillium* spp. is the most common and the most destructive postharvest diseases, occurring on most fruits and vegetables during storage or transport. *Penicillium* spp. mostly enters tissues through wounds (Agrios, 2005).

Fungal pathogens isolated and identified from the decaying crowns of banana were *Colletotrichum musae, A.flavous, A.niger,* and *Rhizopus spp* were frequently isolated from all four districts, *Fusarium* spp. and *Penicillium* spp from three districts out of the four districts (Table 4). Other fungi, such as *Botryodiplodia theobromae, Verticillium theobromae Cephalosporium* spp. and *Ceratocystis paradoxa,* that have been reported to play an important role as causal agents of crown rot in other countries (Finlay and Brown, 1993) were not isolated during this study.

4. Conclusion

The present study reviled that crown rot is the most prevalent and major challenging disease of banana caused by several fungi, which threatened the utilization of banana throughout different parts of the town. Thus, similar research needs to be conducted across different districts of the country. Moreover, extensive researches are justified towards development of safe and cost effective management strategies to reduce the loss due to crown rot of banana.

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Table 1. Prevalence of crown rot of banana in Jimma town

Markets area	Number of Sample	Disease incidence (%)*	Disease prevalence (%)
Bosa kito	10	100	100
Hermata Merkato	10	100	100
Hermata Mentina	10	100	100
Qochi Mendera	10	100	100
Mean		**100**	**100**

*Values are mean of three replications

Table 2. Type and frequency of fungi associated with crown rot of banana in fruit samples collected from markets in Jimma town

Fungi isolated	(%)Frequency of the isolated fungi[1]				Average
	Bosa Kito	Hermata Merkato	Hermata Mentina	Qochi Mendera	
Colletotrichum musae	52.78	44.19	40.48	50.00	46.86
Fusarium spp.	0.00	11.63	11.90	15.00	9.63
Aspergillus flavuos	13.89	20.93	16.67	7.50	14.75
Aspergillus niger	19.44	9.30	16.67	10.00	13.85
Penicillium spp	0.00	9.30	4.76	7.50	5.39
Rhizopus spp	5.56	4.65	2.38	5.00	4.40
Unidentified	8.33	0.00	7.14	5.00	5.12
Total					**100**

[1]Percentage frequency of fungi computed as individual isolates divided by total isolates

Table 3. Colony and microscopic characteristics of fungal isolates associated with crown rot of banana

Fungal isolate	Fungal isolate characteristics	
	Colony	Microscopic
Aspergillus flavus	The fungus was effuse in nature and grow rapidly on potato dextrose agar. Old cultures were olive to lime green (Plate: A and B).	Spores produced were numerous, globose to subglobose and smooth in nature under microscope (Plate:C).
Aspergillus niger	The colony on PDA was initially white and quickly turned black as conidial production started. Its growth produced radial fissures in the agar (Plate: D and E).	Conidia present were numerous, globose and very rough. Immature spores were brown and older spores turn black (Plate: F).
Colletotrichum musa	The fungus had very little mycelia and grew slowly on PDA. Old cultures were dull white to shiny dull orange (Plate: G and H).	Spores were 1-celled and cylindrical with rounded ends under microscope (Plate: I).
Fusarium Spp.	Usually grow rapidly and produce abundant aerial mycelia that initially are off white and become beige or brown with age (Plate: M and N).	Spores were mostly curved or spindle-shaped and had a foot cell. Spores were 3- to 7 septate under microscope (Plate:O).
Penicillium spp.	showed rapid growth, olive green in color, rapidly sulcate to plicate, margins and the balk side of colony was of white in color (Plate: J and K).	Simple or branched conidiophores, metulae, phialides, and conidia are observed. The appearance of the spore head is like that of a brush (Plate: L).
Rhizpus species	Colonies are very fast growing, maturing within 4 days. typically cotton candy like colony, initially white that turns grey to yellowish brown in time. The reverse is white to pale (Plate: P and Q).	Filamentous, branching hyphae that generally lack cross-walls sporangiospores are produced inside a spherical structure, the sporangium. The sporangiophores terminate with a dark, round sporangium that contains a columella and several oval, brown spores (Plate: R).

Table 4. Occurrence of fungal pathogens associated with crown rot banana

Study area	Isolated fungal Pathogen
Bosa kito	*Colletotrichum musae, A.flavous, A.niger, Rhizopus spp*
Hermata Merkato	*Colletotrichum musae, Fusarium spp. A.flavous, A.niger, Penicillium* spp. and *Rhizopus spp*
Hermata Mentina	*Colletotrichum musae, Fusarium spp. A.flavous, A.niger, Penicillium* spp, and *Rhizopus spp*
Qochi Mendera	*Colletotrichum musae, Fusarium spp., A.flavous, A.niger, Penicillium* spp and *Rhizopus spp*

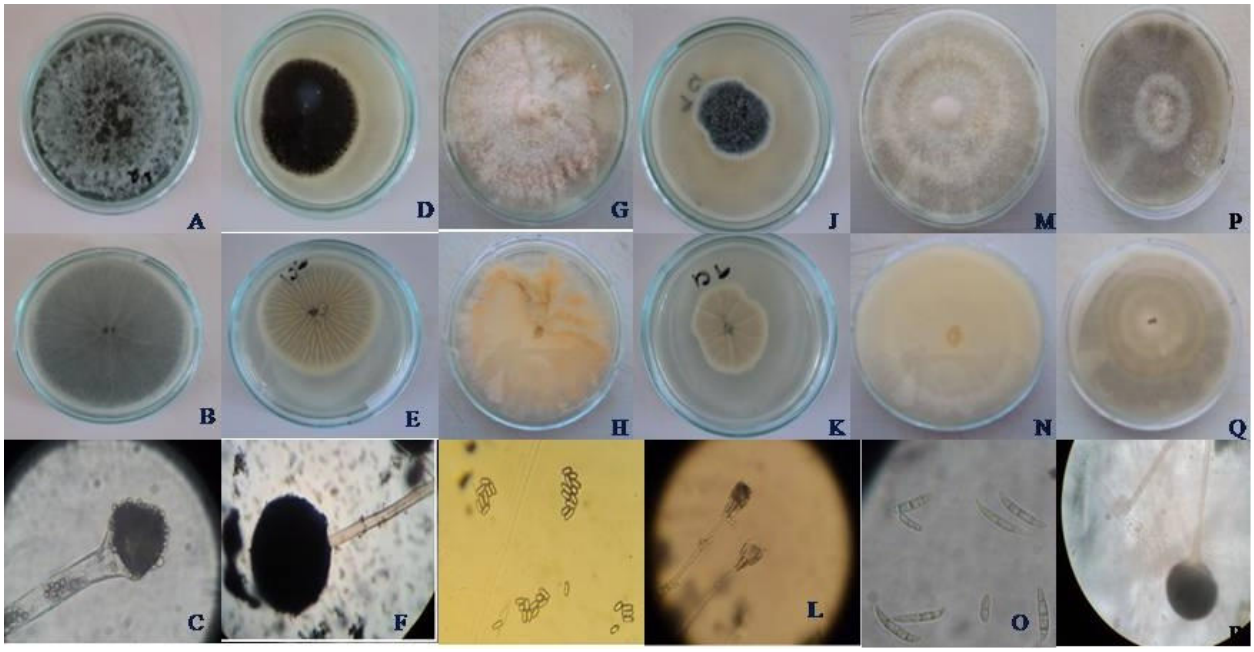

Figure 1. Fungal pathogen associated with crown rot of banana. A-C (*Aspergillus flavus*), D-F (*Aspergillus niger*),G-I(*Colletotrichum musa*) J-L (P*enicillium* sp) M-O (*Fusarium* spp.) and P-R (*Rhizopus* spp.)

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