Survey and Control of Post Harvest Fungal Diseases of Banana (*Musa* spp.) Fruits in Selected States in Sudan

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Dedication

To the Soul of my mother... ...

To my father ...

To my brother...

To my husband...

To my friends...
Acknowledgments

First of all my thanks to Allah, the most gracious, beneficent and merciful, who offered me all things to accomplish this study.

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Abstract

This study was conducted to isolate and identify the causal agents of post harvest diseases associated with some banana lesions. The post harvest diseases of banana fruits in Khartoum local markets which represented different areas of production in Sudan were surveyed. The survey covered seven states including, Khartoum, Kassala, River Nile, Equatoria (Republic of Southern Sudan), Blue Nile, Sennar and White Nile. Isolation of pathogens from infected banana tissues was carried out on PDA using tissue transplanting method. The fungi isolated were *Verticillium theobromae*, and *Trachysphera fructigena* the causal agent of cigar end rot disease, *Colletotrichum musae*, the causal agent of anthracnose disease, *Fusarium moniliforme, F. semitectum, F. oxysporum, Verticillium theobromae, Colletotrichum musae* and *Curvularia* sp. the causal agents of crown rot and *Botrydiplodia theobromae* the causal agent of finger rot. Some other isolated fungi were: *Aspergillus* spp., *Rhizopus* sp., *Cladosporium* sp. and different types of *Yeasts*. The rate of occurrence of post harvest diseases in each state was calculated. Cigar end rot disease was found in higher percentages in all States (The frequencies of occurance were, 60%, 50%, 45% and 40% for the samples from Kassala, River Nile, Equatoria and Khartoum States respectively, and 25% for samples from the remaining states. Other diseases showed variable incidence percentages in the seven states studied. Pure cultures of the fungal isolates were maintained on Potato Dextrose Agar (PDA) medium for morphological studies. The pathogenicity of the fungal isolates was carried out to prove whether these isolates meet Koch's postulates. In this respect, *F. moniliforme; F. oxysporum; F. semitectum; V. theobromae; T. fructigena;* and *C. musae* showed positive results. However, other fungal isolates (*Cladosporium* sp., *Aspergillus* spp. and *Yeast* species) failed to cause infection, and were therefore considered as secondary contaminants.
Two methods of control were used; *in vitro* biological control using either plant extracts or different species of *Trichoderma*.

In case of *Trichoderma* spp., the results showed that *T. viride* and *T. koningii* exhibited maximum inhibition against most tested pathogens with reduction percentages in the range of 39-70%. However, all different species *Trichoderma* showed very low reduction percentages (23-39%) against *B. theobromae*.

In the case of *in vitro* control of rot pathogens using plant extracts, aqueous (hot and cold). Extracts of neem leaves and garlic was tested. Both hot and cold water neem extract showed high inhibition percentages (48% and 60% respectively) against *C. musae*. Cold and hot water extracts of garlic showed 70% and 50% inhibition percentage against *F. moniliforme*, respectively. Neem and garlic extracts recorded their lowest effect against *B. theobomae* and the % inhibition was 7 and 13% in cold and hot water, respectively. It was concluded that the use of *Trichoderma* spp. and the plant extracts could be used to control most of the banana post harvest diseases. Future detailed study is, however, required to determine easy and inexpensive way of application.
الخلاصة

هدفت هذه الدراسة لمسح وعزل ومعرفة مسببات عوامل أمراض مابعد الحصاد المرتبطة ببعض الموز التالف. أمراض مابعد الحصاد بالنسبة للموز في الأسواق المحلية بالخرطوم تمثل انتاج مناطق مختلفة بالسودان. غطي المسح سبع ولايات تشمل الخرطوم، كسلا، نهر النيل، الاستوائية (دولة جنوب السودان)، النيل الازرق، سنار وولاية النيل الأبيض. تم عزل الفطريات في الوسط باستخدام تجربة استزراع النسيج، الفطريات التي تم عزلها شملت Dextrose Agar (PDA) cigar end rot وهي مسببة لمرض Trachysphera fructigena و Verticillium theobromae ، Fusarium moniliforme ، anthracnose Colletotrichum musae و Colletotrichum musae ، Verticillium theobromae ، F.oxysporum ، F.semitectum مسببة لمرض Botrydiplodia theobromae و crown rot و M. musae . عزلت الفطريات أخرى مثل: finer rot وأنواع مختلفة من الخمائر.

(1) برصد معدل ظهور المرض مابعد الحصاد في الولايات ووجد أن معدل أعلى نسبة في كل الولايات والنسب هي 60% و50% و45% و40% للعينات من الولايات ، كسلا، نهر النيل، الاستوائية والخرطوم على التوالي و25% للعينات من بقية الولايات. ظهرت بقية الامراض بنسبة مختلفة في الولايات السبع. حظشت الفطريات النقية المعزولة في الوسط PDA وذلك لدراسة الخصائص المورفولوجية لها. برهنت الدراسة أن الفطريات المعزولة تقبل فرضيات كوكح (Koch's Postulates) بالاجابيات وهـذه T. theobromae F.semitectum F. oxysporum F. moniliforme C. musae , fructigena .

(2) فشلت الفطريات الأخرى المعزولة (Yeast species و Aspergillus spp Cladosporium sp) في الإصابة لذلك تم اعتبارها ملوثات ثانوية.

اجريت طريقة (~ in vitro biological control) أو باستخدام مستخلصات نبات، في حالة الضغط الحيوي في المختبر تم استخدام أنواع مختلفة T. koningii و T. viride من Trichoderma spp وأظهرت النتائج أن
الأغلبية القطرات المختارة بتنقص يتراوح بين 39-70% ومع ذلك أظهرت كل الـ Trichoderma النسبة تثبيط ضئيلة جداً (23-39%) تجاه B. theobromae. أثر عصاره النيم وعصارة الثوم (باستخدام الماء البارد والساخن في كل حالة) لضبط أمراض الموز وسجل مستخلص النيم بالماء البارد والساخن أعلى نسبة تثبيط بلغت 70% و 50% على التوالي. أما مستخلص الثوم فقد سجل تأثيراً أقل بلغ 13.7% في الماء البارد والساخن على التوالي.

خلصت الدراسة بأن استخدام Trichoderma spp. ومستخلصات النباتات لضبط أمراض الموز ما بعد الحصاد يمكن أن يقلل من خطورة هذه الأمراض إلا أن دراسات كثيرة ينبغي أن تتم في المستقبل لابتكار طريقة تطبيق سهلة وقابلة للتكلفة. 

x
CHAPTER ONE

INTRODUCTION

Banana (*Musa* spp.) is a commercially important fruit crop in the world trade. In Sudan, banana is the most popular fruit for its nutritive value, low price and availability all the year round. It is grown in almost every state, with annual production of 74 thousand metric tons (FAO, 2004).

It is grown in many parts of the country, including Kassala State and along the banks of the Blue Nile, mainly in Damazin and Sennar, Gezira and and Khartoum States. It’s also grown in some parts of Darfur. About 87% of total production comes from three states namely Sennar, Blue Nile, and Kassala. Kassala State is the main area for banana production (35%) followed by Blue Nile (25%), Gezira, Sennar and Khartoum States (40%) (Albashir and Imam, 2010).

Banana is one of the most important world crops. The fruit is of high nutritional value. It fits well with the recommendations of the Select Committee of the United States on Nutritional and Human Needs for increased consumption of foods low in fats (Anon, 1977). Banana has a good content of vitamin A, all parts of the plant are conspicuously rich in potash (Purseglove,1972). It has been widely used as an element in nutrition for people suffering from obesity due to its easy assimilation.

Beside primary use as a dessert fruit and a stable starch, banana provides other products like fibers, wrappers and confectionery; it also functions as
medicine, keeps the nervous system healthy, increases the production of red blood cells and regulates hormonal activities in the blood (Gane, 1963).

Dwarf Cavendish is the predominant cultivar in Sudan which in spite of its locally preferred taste and flavor, it has short fingers and a poor keeper and shipper traits which is the major reason of its unacceptability in the world markets. Recently, new banana cultivars such as Granien and William have been released from the Sudan National Variety Release Committee targeting the export markets.

Most of the after bruises ripening are due to harvest and transport problems. Post harvest losses of bananas are about 35% (Albashir and Imam, 2010). The most important banana diseases in the world are post - harvest market diseases of banana fruits caused by fungi such as crown rot, cigar-end rot, finger rot, anthracnose and others. Black leaf streak or black Sigatoka caused by the fungus Mycosphaerella fijiensis is the most serious disease affecting the foliage of banana. Fusarium wilt or Panama disease is caused by Fusarium oxysporum f. sp. cubense. Virus diseases include bunchy top, banana mosaic, banana streak and bract mosaic (Crop Protection Compendium, 2005).
The objectives of the present study were:

1- To survey and diagnose post harvest diseases of banana fruits which are transported to Khartoum markets from different areas of production in the Sudan.

2- Isolation, characterization and identification of the causal fungi, and assessment of their pathogenicity.

3- Evaluate the effectiveness of three *Trichoderma* species viz, *T. viride*, *T. harzianum* and *T. koningii*, as bio-control agents against detected pathogenic fungi.

4- Evaluate the effectiveness of cold and hot water *Azadirachta indica* and *Allium sativum* extracts as control agents against detected pathogenic fungi.
CHAPTER TWO

LITERATURE REVIEW

2.1. The plant and its importance:

Banana is an important fruit crop of the world which is cultivated over an area of more than four million hectares and its annual production is more than seventy million tons (FAO, 2006). Bananas are now grown pantropically in one hundred and thirty countries which is more than any other fruit crop. Edible *Musa* spp. originated in Southeastern Asia and spreaded westwards, along the major trade routes that transported other fruits.

Most of the bananas are used as fresh fruit but nowadays bananas are used in many forms other than fresh consumption. Banana puree, ice cream, baked desserts and dried sugary slices are very popular among the people. As with most fruits, the fermented juices of banana are made into beer and wine.

Banana varieties include Cavendish, Latundan, Lakatan, Inarnibal, Amas, Bungulan, Pitogo, Murado, Inabanako and Senorita. Most banana cultivars have 33 chromosomes (2n = 3x). These triploid genotypes are virtually or completely sterile and develop their fruits by vegetative parthenocarpy. Diploid landraces and tetraploid cultivars (mostly artificial hybrids) are also cultivated.
Banana and the closely related plantain, or cooking banana, occur in a
great diversity of forms resulting from the mixing of *Musa acuminata* and
*Musa balbisiana* in their genetic makeup. Such forms are usually large,
perennial, monocotyledonous herbs, 6–30 ft (2–9 m) tall arising from
large, underground rhizomes (Nelson, 2008).

**2.2. Banana Diseases:**

Like many other crops, pests and diseases have significantly affected
*Musa* cultivation all over the world, these diseases are either field
diseases or post harvest diseases.

**2.2.1. Field diseases:**

**2.2.1.1. Panama disease:**

The history of the *Fusarium* wilt (Panama) disease of banana and
plantains caused by *Fusarium oxysporum f. cubense* has been
comprehensively reviewed by Stover (1962), Ploetz (1990) and more
recently by Ploetz and Pegg (2000). Panama Disease or Banana Wilt,
originates in the soil, travels to the secondary roots, enters the corm only
through fresh injuries, passes into the pseudostem; then, beginning with
the oldest leaves, turns them yellow first at the base, secondly along the
margins, and lastly in the center (Wardlaw, 1972). The most
characteristic symptoms of the disease are the brown-reddish
discoloration of the internal vessels of the pseudostem.
The oldest leaf sheaths can show brownish streaks (Ploetz and Pegg, 2000). It is reported by Wardlaw (1972) that this disease has seriously affected banana production in Central America, Colombia and the Canary Islands. Also, the disease has been reported in West Africa, South Africa and East Africa (Stover, 1990).

The use of resistant genotypes has proved to be the main measure of control, in disease free areas preventative and quarantine procedures should be implemented to avoid the entry of the pathogen but there are no effective measures of chemical control (Pérez-Vicente, 2004).

2.2.1.2. Sigatoka disease:

This disease is caused by *Mycosphaerella* spp. Sigatoka disease is named after a valley in Fiji where the problem first attracted attention in the early part of the century (Meredith, 1970). It is now found in most of the humid tropics in Africa, the Americas and Asia, and continues to spread to the few remaining areas that are free of the disease (Ploetz and Mourichon, 1999). Black Sigatoka and the closely related yellow Sigatoka are the primary leaf spot diseases of banana. They are caused by two ascomycete fungi, respectively, *Mycosphaerella fijiensis* Morelet (anamorph: *Paracercospora fijiensis* (Morelet) Deighton) and *M. musicola* Mulder (anamorph: *Pseudocercospora musae* (Zimm.)), which produce similar symptoms on banana (Ploetz, 2000).
Black sigatoka (M. fijiensis) is considered as one of the most damaging and costly diseases of banana (Marín et al., 2003). The socio-economic impact of black sigatoka continues to increase as the pathogen reaches new areas. As bananas are cultivated in more than 100 countries throughout the world, the impact of black sigatoka has also increased as it becomes more difficult to be controlled (Marín et al., 2003). Therefore, the ability to assess world-wide geographical distribution of the worse disease of banana under climatic change has practical implications on climatic zoning of the crop, establishment of agricultural government policies and adequate disease management.

Sigatoka, also known as leaf spot, is a foliar disease characterized by well defined, necrotic, and generally elongated spots. This fully developed spots persist after the leaf desiccates (Kranz et al., 1977). The disease is different from others in that the causal organism is not itself present in the fruit but yet has profound effects on the fruit development (Stover, 1980). Sigatoka is essentially a leaf-spotting disease which can cause pre-mature death of large areas of the plant's leaf surface. Photosynthesis is thereby drastically reduced, sometimes to the extent that fruit does not mature at all. According to Stover (1980) this disease is controlled by strict quarantine laws to prevent the spread of the causative fungi. In countries where the disease is established, control is by cultural and chemical methods. It is reported by Cronshaw (1984) that frequent application of
fungicide may be needed and, in order to delay the appearance of tolerant fungal strains, different fungicides are used in rotation. In view of the seriousness of this disease, it is important to breed for resistant banana cultivars (Stover, 1980).

2.2.2. Post harvest diseases:

2.2.2.1. Anthracnose:

Anthracnose is one of the important post harvest diseases of banana known in all producing countries (Zakaria et al., 2009). It is caused by the fungus *Colletotrichum musae* (Berk. & Curt.) V. Arx (Smoot et al., 1971; Prusky and Plumbley, 1992). The fungus can infect banana fruits at any time during the growing season in the field. Banana anthracnose usually starts as quiescent infections on green fruit in the field; however, successful penetration of the fungus is restricted by accumulation of phytoalexin as the fruits ripen (Jeger et al., 1995, and Turner, 1997). Therefore, symptoms generally can be seen only in over ripe fruits. Ismail (2004) isolated *C. musae* from Sudanese banana infected with anthracnose.

Lapeyre and Mourichon (1997) reported that anthracnose which was caused by *C. musae* was the main disease affecting banana after harvest; flower parts are the main inoculum source for the fungus.

There are two types of symptoms, resulting from different modes of infection, non latent and latent infections (Meredith, 1971, Snowdon,
1990 and Ploetz et al., 1994). The non latent infection occurs in small wound starting from harvest and continuing to develop then after harvest without a dormant period.

*C. musae* is the most important pathogen on wounded green and ripe banana fruits (Meredith, 1960; Stover and Simmonds, 1987). Occasionally, the fungus invades necks of green fingers when damaged by flexing (Wardlaw, 1972). Lesions are sunken and covered with salmon-coloured acervuli. Infections stimulate ripening of fruits and lesions elongate with ripening. On ripening fruits, sunken brown spots develop with orange acervuli (Stover and Simmonds, 1987).

Shillingford and Sinclair (1977) reported that the large lesions are the result of infection following physical injury, the fungus gaining entry via wounds sustained during harvesting and handling.

Peacock and Muirhead (1974) described that the circular spots are the result of pre-harvest infections initiated in uninjured immature fruit. Kaiser and Lukezic (1966) showed that the optimum temperature for growth, sporulation and conidium germination in *C. musae* is from 27 to 30 °C.

Snowdon (1990) cited that strict sanitation is necessary in plantation and pack house, in order to minimize the number of spores available for infection. Griffee and Burden (1974) showed that the fruit must be harvested at the correct stage of maturity and handled carefully to prevent
injury. The same authors presented that in situation where chemical control is necessary, post-harvest treatment with a systemic fungicide is more effective than pre-harvest spraying.

Liu et al. (1997) showed that post harvest heat treatment can be used for quarantine of imported fruits and for reducing fruit decay and physiological disorders during storage. The treatments may involve use of hot water (46-55 ºC) for 10 – 60 min. Treatment of banana with hot water and Benlate (500mg/litre) controlled anthracnose (C. musae) during storage. Ahmed and Mohamed (1988) described that of the 28 species of fungi isolated from the surface of banana fruits, Gliocladium sp., Pencillium sp. and Trichoderma spp. were antagonistic towards C. musae in dual culture. They reported that culture filtrates of the isolates inhibited the growth rate, germination of conidia and germ tube length in vitro. Spores from C. musae cultures irradiated with gamma ray 4KGY did not germinate. Burden (1968) found that complete control of latent infections was obtained by dipping green banana in hot water (55ºc) for 2 min. before ripening.
2.2.2. Crown rot:

Crown rot is one of the most important post harvest diseases of banana and plantain. It is a characteristically complex disease caused by several fungi sometimes in association with other microorganisms such as bacteria (Lukezic et al., 1967; Meredith, 1965 and Snowdon, 1990). Crown rot of banana once caused a serious problem for post harvest fruits during transit (Greene and Goos, 1963 and Papaisri Pitakaivan, 1986). The most common pathogens associated with crown rot are *C. musae* (*Gloesporium musarum*), *F. roseum*, *F. semitectum* and *B. theobromae*. Other species including *Cephalosporium* sp., *V. theobromae*, *Ceratocystis paradoxa* and *Phomopsis* sp. have been associated with the crown rot complex (Ploetz et al., 1994 and Snowdon, 1990). In addition, more than a dozen other fungi have been found in crown rot affected tissues (Ploetz et al., 1994). The most common pathogens associated with crown rot fungi of banana were isolated and identified from fruit obtained from Mexico, Guatemala, Cost- Rica and Ecuador in 1993. *F. semitectum*, *Penicillium* spp., were isolated more frequently. Other fungi isolated were *F. moniliforme* and *G. roseum* (Martin et al., 1996).

Lapeyre and Mourichon (1997) reported that crown rot which was caused by *Fusarium* spp. is the main disease affecting banana after harvest and the contamination is brought from the field during flowering.
Mesturino and Ragazzi (1988) isolated some fungi from banana fruit which caused crown rot and these fungi were *C. musae*, *F. sporotrichioides*, *Fusarium* spp., *Pestalotia* spp., *Cladosporium* spp., *Acremonium* spp. and *Alternaria* sp..

Ogundero (1987) isolated repeatedly 8 fungi from Nigerian banana randomly collected from shops and which were infected with crown rot. These fungi were *C. muase*, *F. solani*, *C. paradoxa*, *F. roseum*, *F. moniliforme*, *F. oxysporum*, *B. theabromae* and *V. theabromae*. Ismail (2004) isolated *C. musae*, *V. theabromae* and *Fusarium* spp. from Sudanese banana infected with crown rot.

Greene and Goos (1963) reported that *Deightoniella torulosa* and *Ceratocystis paradoxa* cause severe crown rot. Ogawa (1970) showed that in Africa and Asia *B. theobromae* and *C. paradoxa* are frequently isolated from banana showing crown rot symptoms. Griffee (1976) reported that in Central America and the Caribbean the most prevalent fungi associated with crown rot are *C. musae*, *F. pallidoroseum* and *V. theobromae*.

Slabaugh and Grove (1982) reported that in the last three years *Fusarium roseum*, *F. semitectum* and *Acremonium* sp. have been the principal fungal pathogens responsible for this disease. These pathogens exist in banana fields on dead banana leaf or inflorescence tissues. They disperse by wind and water, and by some insects, birds, and rats (Meredith, 1971;
Slabaugh and Grove, 1982 and Nelson, 2008). Meredith (1971) recorded that the spores of fungi are dispersed either by wind or rain splash, and impinge on all parts of developing bunch.

Abdel-Sattar et al. (1977) reported that while, grey or pink mould may form on the surface of the cut crown, infected tissue turns black and the rot may advance into the finger stalks, causing the fingers to drop off when handled. Greene and Goos (1963) reported that when the harvested fruit is cut into hands and washed, the newly exposed tissue is vulnerable to infection; spores which have accumulated in the washing water can be drawn several mm into the wound.

Badger (1965) showed that 86 % or more relative humidity was necessary for germination of conidia of most crown rot fungi. Ungerminated conidia remained viable for several months under extremes of relative humidity and temperature.

Knight et al. (1977) reported that the crowns of green banana bands treated at harvest with bneomyyl were inoculated with *F. semitectum* and *C. musae*. During ripening rots developed at the points of inoculation and there was little contamination by other organisms. The control usually remained entirely or fairly free from rotting followed inoculation with either fungus or with both did not differ significantly. *F. semitectum* is therefore considered to be a primary wound pathogen.
Meredith (1971) reported that preventive measures begin in the plantation with regular removal of leaf trash, hygiene is also important in the packing station, and washing water should be changed frequently before it becomes heavily contaminated with spores. Also, he reported that dehandling should be done carefully with sharp knife so as to avoid leaving a ragged cut. In some contents ‘crown beveling’ has proved beneficial, entailing a further trimming of the crown after the washing process.

Shillingford and Sinclair (1977) stated that it is of great importance to minimize the time between cutting and cooling the fruit, especially if a long voyage is scheduled. Rapid reduction of temperature serves to slow down development of crown decay.

Many chemicals have been suggested for crown rot control. Dipping hands in Nabam (disodium ethylenebisdithiocarbamate) was moderately effective during the period of cool storage, but did not control disease through to the ripening stage. Formaldehyde (5%) gave good control but was phototoxic. Maneb (Manganese ethylenebisdithiocarbamate and Zineb (Zinc ethylenebisdithiocarbamate) at 0.5 to 1.0 percent usually gave good control.

Attempts to surface - sterilize bunches, before dehandling, were made by spraying with sodium or calcium hypochlorite solutions, or with chlorine water. Spraying was contained for 2 minutes and then washed off with
water. Preliminary commercial shipments indicated that this type of treatment by chlorine was sometimes as good as a Maneb dip for controlling crown rot (Meredith, 1971).

Burden (1967) found considerable reduction of crown rot when naturally infected hands were dipped for a few seconds in thiabendazole (TBZ) suspensions containing 200 to 800 ppm of active ingredient. Excellent control was obtained when hands were thoroughly washed in 0.5 percent calcium hypochlorite, rinsed for 20 minutes in water and then dipped in 300 to 400 ppm TBZ for 2 to 3 minutes.

Laboratory tests by Ogawa et al. (1968) in Taiwan showed that the level of control provided by a dip in 1600 ppm benomyl (50 percent 1-butylcarbamoyl-2-benzimidazole carbamic acid, methyl ester) was better than that by captan. Burden (1968) found that dipping green bananas in hot water (55 ºC) for 2 min resulted in some, but not complete, control of crown-rot.

2.2.2.3. Cigar- end rot:

Cigar end rot is an economically important disease in central and West Africa (Ploetz et al., 1994). It can be caused by either V.theobromae (Turc ) ,( Mason & Hughes) or T.fructigena (Tabor & Bunting) (Ploetz et al., 1994). T. fructigena is present in Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea, Ivory Coast, Democratic Republic of Congo, Gabon, Ghana, Guinea, Madagascar, Nigeria and
Sierra Leone (European and Mediterranean Plant Protection Organization, EPPO, 2005).

Maramba and Clerk (1974) found that *T. fructigena* can cause a destructive rot in the plantations of West and Central Africa while *V. theobromae* is more wide spread occurring in most banana growing regions including India, Iran, Egypt, South Africa and the Canary Islands. Ismail (2004) isolated both *V. theobromae and T. fructigena* from banana fruits showing cigar end rot symptoms, in Sudan.

Snowdon (1990) reported that one or all fingers may be affected by a tip-end rot which starts with localized darkening and wrinkling of the skin. In *Trachysphaera* rot the surface of the lesions becomes covered with white spores which later turn pink or brown as they mature giving the fruit finger tip the greyish ashy colour usually associated with cigar end rot; internally the pulp may undergo a wet rot or, in the absence of secondary organisms, may become dry and mummified. In *Verticillium* rot the tissue is characteristically dry and fibrous, and the spores are grey and powdery. In both diseases the symptoms bear a resemblance to the ashy end of burnt cigar (Ploetz *et al.*, 1994 and Snowdon, 1990).

Meredith (1965) reported that these fungi tend to be prevalent during wet or humid growing seasons, and air-borne spores can cause infection of the dying flower parts.
The chief method of controlling this disease is through frequent manual early removal of dead flower parts, followed by bagging of the developing fruits. Spraying with fungicide may be necessary in some seasons. Packing stations and ripening rooms need to be kept clean to minimize the chances of post harvest infection (Jones and Stover, 2000; CPC, 2007 and Snowdon, 1990).

2.2.2.4. Finger rot:

Finger rot is caused by the fungus *B. theobromae* Pat. This fungus invades wounds on the fruit skin (Dadzie and Orchard, 1997). The fungus penetrates the pulp and rots the entire fingers and can pass to neighboring hands. Rotting fingers ripen more rapidly and can trigger pre-mature ripening in an entire box. Rotting usually begins at the tip of one of the finger or at a wound site. The decay spreads uniformly and causes a brownish black discoloration of the peel and a softening of the pulp. The affected area of the peel becomes wrinkled and encrusted with minute black bodies (pycnidia). The pulp is reduced to a soft (or semi-liquid state), rotten mass, and a dark grey mould grows on the peel surface when the humidity is high. The rate of disease development increases during fruit ripening and can spread to adjacent fingers. Infected clusters tend to ripen prematurely, and fully matured fruit is the most susceptible to infection (Willamson and Tandon, 1966, Ogawa, 1970, Snowdon, 1990 and Ploetz *et al.*, 1994). The disease can be held in check by minimizing
fruit injury, by treatment of fruits with systemic fungicide and by rapidly reducing fruit temperature after harvest (Snowdon, 1990).

Other diseases of importance are rhizopus rot due to *Rhizopus oryzae* and *R. stolonifer* reported from different states of India (Bilgrami et al., 1979, 1981). Black mould rot due to *Aspergillus niger* (Thakur and Chenulu, 1970 and Mandal and Dasgupta, 1983). Black rot caused by *Cladosporium cladosporoides* are also reported from India (Manoharachary and Rama Rao, 1989).

### 2.3. *Trichoderma* spp. as a bio-control agent

*Trichoderma* spp. are fungi that are present in nearly all soils and other diverse habitats such as decaying wood. According to Harmann (2005) the fungi are the most prevalent fungi in soil. They are favoured by the presence of high levels of plant roots, which they colonize readily. In addition to that *Trichoderma* spp. are able to attack, parasitize and otherwise gain nutrition from other fungi. These abilities turn the fungi into a reputable bio-control agent.

#### 2.3.1. Taxonomy:

The sexual life-form of the fungi of the genus *Trichoderma* is extensively unknown and therefore, the fungus has been classified in the class of *Deuteromycotina*. Reproduction of the ubiquitous genus is by conidia which are produced from highly branched conidiophores. These are arranged in irregular verticils, with sub-terminal phialides. The shape of
the phialides is typical for *Trichoderma* sp. and is the crucial factor for the identification of the fungus (Pitt and Hocking, 1985). *Trichoderma* colonies spread rapidly and have a loose textured mycelium which characteristically develops irregularly, with tufts or isolated patches. The mycelium has a white transparent colour. The small conidia are in general smooth, green coloured and develop after a short time of incubation. That is the reason why the mycelium turns green after few days.

**2.3.2. The role of mycoparasitism**

As aforementioned, *Trichoderma* spp. are used as bio-control agents against phytopathogenic fungi and bacteria. *Pythium*, *Phytophthora*, *Rhizoctonia*, *Fusarium* and *Verticillium* are fungi against which *Trichoderma* spp. have already made an impact (Korting, 2001). The reasons for this use are certain antagonistic attributes which most fungi out of the genus *Trichoderma* own. The most important mechanisms are, according to Wantoch-Rekowski (2004), mycoparasitism, antibiosis and competition. *Trichoderma* spp. are able to enter into the mycelium of other fungi. Therefore, the fungi wind around the host’s mycelium and build hyphae which break into the host’s mycelium. *Trichoderma* produces enzymes such as cellulases and chitinases which are able to destroy the cell walls of other fungi or inhibit the germination of other spores. Some *Trichoderma* strains produce antibiotics which can control bacterial phytopathogens.
According to Wilke (2001) *Trichoderma* produces some compounds such as hormones and enzymes which support the growth of the cultured plants. Apart from parasitism and antibiosis *Trichoderma* sp. can compete with other fungi against nutrients and space.

2.4. Control of post harvest diseases of banana:

2.4.1. Biocontrol with *Trichoderma* species:

Normally, fungicides are the prime means of controlling post-harvest diseases (Eckert *et al.*, 1994). Crown rot of banana is controlled commercially by submerging clusters of bananas in solutions of thiabendazole (TBZ), imazalil or benomyl (Sepiah and Nik Mohd, 1987; Krauss *et al.*, 1998; Aked *et al.*, 2001). The use of synthetic chemicals to control post-harvest rots and deterioration has been limited due to their potential carcinogenicity, teratogenicity, environmental pollution, effects on food and other side-effects (Unnikrishnan and Nath, 2002 and Marin *et al.*, 2003). Synthetic fungicides can also leave toxic residues (Zahida and Masud, 2002). These drawbacks of chemical control have increased interest in alternative control methods, particularly those that are environmentally safe (Conway *et al.*, 1991, Sugar *et al.* 1997 and Wilson *et al.*, 1997). Biological control is a possible alternative to fungicides in a post-harvest environment, where temperature and relative humidity are controlled (Spotts and Sanderson, 1994). Post-harvest bio-control is also a good idea because harvested fruits are easy to treat biologically. In
banana crown rot, the pathogen often infects banana hands through wounds created during dehanding after harvest, indicating that colonization by antagonists is also a likely means of control.

The success of *Trichoderma* strains as biological control agents (BCAs) is due to their high reproductive capacity, ability to survive under highly unfavourable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi, and efficiency in promoting plant growth and defence mechanisms (Benitez *et al*., 2004).

Most approaches to the biological control of post-harvest diseases have examined single bio-control agents against diseases caused by single pathogens. Examples are, on banana, control of *C. musae* (anthracnose) with *Trichoderma viride* (Golam *et al*., 1998) and, on yam, control of a post-harvest disease caused by a complex of pathogens (*A. niger, L. theobromae* and *Penicillium oxalicum*) using *T. viride* (Okigbo and Ikediugwa, 2000). The antagonistic potential of *Trichoderma* spp. against various post-harvest pathogens has also been studied to counter green mould of citrus (De Matos, 1983), anthracnose of banana (Golam *et al*., 1998), post-harvest pathogens of rambutan (Sivakumar *et al*., 2001), *B. cinerea* on apple and fruit (Tronsmo and Dennis 1997).
2.4.2. *In vitro* control of banana post harvest diseases using plant extracts:

Pollution problems in the environment and toxic effects of synthetic pesticides on non-target organisms have prompted investigations on exploiting pesticides of plant origin. Natural plants products and their analogues are an important source of agricultural pesticides (Grainge and Ahmed, 1988; Chandrasekaran and Gunasekaran, 2007). These are used in the control of insect pests (Emosaire and Ukeh, 1996), plant diseases (Al-Abed *et al.*, 1993) and bird repellants (Mason and Mathew, 1996). Furthermore, pesticides of plant origin are cheaper, readily available and cost effective in developing countries where synthetic fungicides are scarce, often adulterated and expensive for resource poor farmers. Through *in vitro* investigations, Bankole (1994), Obagwu *et al.* (1997), Adetogun and Atayese (2006), Eziashi *et al.* (2006), Muhamad and Mustapha (2006), Akpa and Amodu (2006), Ogbebor *et al.* (2007) and Nduagu *et al.* (2007) confirmed the fungicidal potential of extracts of *Azadirachta indica* (neem), *Khaya senegalensis* (mahogany), *Allium sativum* (garlic) and *Zinziber officinale* (ginger) on *Alternaria solani*, *Colletotrichum* spp., *F. oxysporum*, *R. solani*, *P. corylophilim*, *C. paradoxa*, *Drechslera heveae*, *Xanthomonas oryzae* and *Erwinia carotovora* which are all pathogenic on valuable crop plants causing important diseases. Alabi (1986) reported the efficacy of both cold and
hot water extracts of *A. indica* on *Dothiorella dominica*, the causal agent of mango soft rot. Amadioha (2000) obtained lower rice blast (*Pyricularia oryzae*) incidence and severity with cold water extracts of *A.indica* compared to hot water and alcohol extracts. Markson *et al.* (2004) and Madunagu *et al.* (2004) also reported the superiority of cold water extracts of *Piper guineensis*, *Aframum melegueta*, *Jatropha curcas*, *Ageratum conyzoides* and *Emilia sanchifolia* over their ethanolic extracts. The cold water extract of neem leaf and seed, garlic bulb, mahogany seed, ginger rhizome and shea butter leaf was tested in the control of *Curvularia eragrostidis* isolated from pearl millet *in vitro* and *in vivo*, cold extract of each tested plant material reduced mycelial growth, sporulation and spore size of the pathogen better than hot water extracts (Zarafi *et al.*, 2004).
CHAPTER THREE
MATERIALS AND METHODS

3.1. Samples collection:
Infected banana fruit samples showing various symptoms of post harvest
diseases were collected from the markets of the different states
(Khartoum, River Nile, Kassala, Equatoria (Republic Southern Sudan,
R.S.S.) Sennar ,White Nile, and Blue Nile) for two consecutive years and
brought to the laboratory in separate clean polythene bags for further
studies. As soon as the infected fruits were brought in the laboratory, the
symptoms were critically studied.

3.2. Isolation of fungi:
In order to differentiate between physical and pathological damage of
banana fruits the small pieces of the infected peels of the banana samples
were incubated in Petri- dishes in order to encourage fungal growth using
tissue transplanting method. From each area a random sample containing
20 fruits was taken and tested.

3.2.1. Tissue transplanting method:
Small pieces (2-4mm²) of infected tissues of the peels of banana fruits
from each of the stalk, middle and the end of the fruit were disinfected by
dipping in 4% sodium hypochlorite solution for 3 min. and plated on
three layers of moistened sterilized filter papers (9 cm in diameter). The
plates were then incubated at room temperature (Plate 1).
Plate 1: Tissue transplanting method

a: Isolation of fungi from infected tissue

b: Isolation in culture medium
The Petri-dishes were examined daily for the development of fungal infections. After 7 days any mycelial growth observed was examined under stereoscopic binocular microscope, with magnification up to 50X to monitor fungal development on their host and the incidence of fungi was then noted.

3.2.2. Isolation of fungi in culture media:

Pieces of tissue from the margin between infected and healthy tissues were first surface sterilized using 70% ethanol for one minute, rinsed in sterilized distilled water. Small pieces of infected tissue were taken from just under the fruit skin and were plated in the Petri-dishes containing Potato Dextrose Agar (PDA) medium amended with chloramphenicol (500mg/l) to suppress bacterial growth. Three replicates were prepared for each fruit lesion. The inoculated plates were then incubated at 25°C. The plates were then examined daily for 7 days for the development of fungal growth. Where a clear growth was observed, purification was done by repeated sub-culturing on PDA plates.
3.2.3. Identification:

Morphological characteristics such as the shape, colour and the size of conidia for each isolated fungus were examined under a stereoscopic binocular microscope to a magnification up to 50X after incubation at 25°C. Slides for microscopic observation were either prepared in lactophenol, in water in the case of dark-coloured fungal spores or in lactophenol cotton blue in the case of hyaline spores. Permanent slides were prepared by removing small cylinder of agar by a cork borer, and inserting on the surface of a thin layer of Corn Meal Agar (CMA) in a Petri dish. The top of the agar cylinder is inoculated with the fungal growth and covered with sterilized cover slip. After few days, fungal growth on the cover slip was gently mounted in lactophenol or lactophenol cotton blue according to the colour of the fungal spores and examined under Oil Immersion. Isolated Fungi were identified according to Meredith (1965) and Snowdon (1990).

3.2.4. Single spore isolation:

The Petri-dishes containing the PDA cultures were placed under a stereoscopic binocular microscope placed in a laminar, spores of the fungi were transferred to a large square drawn on the back of the Petri-dish containing an isolation medium (CMA). Individual spores were transferred from the large square to smaller ones drawn at the back of the Petri-dish near the large square. The Petri-dishes were incubated at 24°C.
for 24 hours. When the individual spores germinated a square was cut in the agar around each germinating spore using a sterile scalpel. The cut square containing the germinating spore was transferred to a Petri-dish containing filtered (CMA). Cultural characteristics and mycelial growth on the media was observed after incubation for 7 days at 25°C. The different isolated fungi were described and photographed.

3.3. Proof of pathogenicity:

The pathogenicity of various fungi isolated from infected banana fruits was carried out according to Koch’s postulates. Pathogenicity was tested on healthy banana. Uninjured fruits were collected and used to prove whether the isolated fungi are capable of inducing disease symptoms on healthy fruits similar to those observed on infected samples.

3.3.1. Inoculation and incubation:

The pure cultures of the isolated fungi from fruits were used to inoculate sites at a slightly scratched healthy banana fruit peel. Injured fruits were inoculated with mycelial plugs (5 mm) prepared from 7-day-old mono spore culture of each isolate. Two replicates were prepared from each fungus. The inoculated fruits were incubated at room temperature (28°C ±2) for 5 days.
3.4. Control:

Five fungal isolates viz. *V. theobromae*, *F. oxysporum*, *F. moniliforme*, *C. musae*, and *B. theobromae* which are known to cause different banana diseases were used as test organism to evaluate the effectiveness of three *Trichoderma* species and two plant extracts as control agents.

3.4.1. *In vitro* biological control:

Cultures of pathogens and those of three antagonistic fungi viz *Trichoderma viride* Pers. ExGray, *T. harzianum* and *T. koningii* Oudem, (obtained from the culture collection of Plant Pathology laboratory of the Plant Protection Directorate, Khartoum) were maintained on PDA medium. The effectiveness of the three antagonistic fungi against mycelial growth of *F. moniliforme*, *F. oxysporum*, *V. theobromae*, *B. theobromae* and *C. musae* was studied by Dual Culture Technique (Rama Bhadra Raju and Krishna Murthy, 2000). For each test, a 4 mm diameter mycelial disc from a 7-day-old culture of each of the tested fungi was placed on the agar surface of a nine cm Petri dish one cm from the edge of the dish. A four mm diameter mycelial disc from an actively growing *Trichoderma* culture was then placed on the agar surface opposite to the target pathogen. Four replicate plates were maintained for each treatment and a control set was also prepared. Plates were incubated with the experimental controls (pathogen without *Trichoderma*) at 28±2°C for 4 days, and the radial growth (mm) of the pathogen mycelium was
recorded. Percent inhibition of mycelial growth of the pathogen was calculated as follows:

Inhibition % = \frac{C - T \times 100}{C}

C: the radial growth measured for the control

T: the radial growth measured for the Trichoderma species

3.4.2. In vitro control of rot pathogens using plant extracts:

Neem leaves and garlic bulbs were air-dried, powdered separately using mortar and pestle. Twenty grams of each powder were mixed with 5 ml 95% ethanol for 5 minutes for surface sterilization. The cold water extracts were obtained by infusing 20 g of each powder in 100 ml of sterile water for 24 hours. The hot water extracts were prepared by mixing twenty g of each sterilized plant material in 100 ml of sterile water and each flask was placed in a water bath at 90°C for 1.5 hours (Zarafi and Moumoudou, 2010). The suspensions (cold and hot water) were filtered separately through a double layer of sterile muslin cloth. Ten milliliters of each suspension were mixed in 100 ml of PDA to obtain Potato Dextrose plant extract agar (PDA–plant extracts) mixture. Twenty ml of PDA – plant extract were dispensed in Petri dishes and allowed to solidify. Inoculum discs of 5 mm diameter obtained from the edge of a seven day old culture of each tested isolate on PDA were pressed with face downwards at the centre of each of the different PDA-plant extract plates separately. PDA without any plant extract served as a control,
while PDA with Byfidan served as a positive control. Three replicates for each treatment were prepared and all plates were incubated at 28±2°C for 7 days and the radial growth (mm) of the pathogen mycelium was recorded. Percentage inhibition of mycelial growth of the pathogen was calculated as follows:

$$\text{Inhibition} \% = \frac{C - T \times 100}{C}$$

C: the radial growth measured for the control

T: the radial growth measured for each extract in the tested plates.
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Samples collection:

Disease symptoms observed on the collected banana samples can be classified visually (Plate 2) as follows:

1. Lenticular lesions with black or brown colour on the surface of the peel (Plate 2a). These symptoms were described as anthracnose (Snowdon, 1990).

2. Small black sunken spots, while some lesions were elongated, others showed a white growth on the peel (Plate 2b). These lesions were observed on the stalk of the fruit and were described as crown rot (Snowdon, 1990).

3. The surface of the tip ends of some fruits were covered with ashy colour and the tissues were dry (Plate 2c). This is described as cigar end rot (Snowdon, 1990).

4. Rotting usually begins at the tip of one of the finger or at a wound site. The decay then spreads uniformly and causes a brownish black discolouration of the peel and a softening of the pulp. The affected area of the peel becomes wrinkled and encrusted with minute black bodies (pycnidia) (Plate 2d). These symptoms were described as finger rot (Ogawa, 1970; Williams and Tandon, 1966; Snowdon, 1990 and Ploetz et al., 1994).
Plate 2: Symptoms of various banana diseases

a: anthracnose
b: crown rot
c: cigar end rot
d: finger rot
4.2. Isolation of fungi:

4.2.1. Tissue transplanting method:

After the 7th day of incubation of small pieces of infected tissues of the peels of banana fruits, fungal mycelial growth was observed on the peels. The fungi were isolated and identified and were recorded. The fungi recorded were *V. theobromae*, and *T. fructigena* and were isolated from the peels showing symptoms of the cigar end rot disease, *C. musae*, was isolated from the peels showing symptoms of anthracnose disease, *F. moniliforme, F. semitectum, F. oxysporum, V. theobromae, C. musae*, and *Curvularia* sp. were isolated from the peels showing symptoms of crown rot, *B. theobromae* was isolated from peels showing symptoms of finger rot disease and *Cladosporium* sp., *A. niger, A. flavus, A. terreus, Yeasts* and *Rhizopus* sp. were isolated from peels showing other rots (Table 1).

As shown in Table 2 *V. theobromae*, the causal agent of cigar end rot was detected in samples collected from all states. *T. fructigena*, the causal agent of cigar end rot, was detected in samples from Khartoum, Equatoria and Nile States, *C. musae*, the causal agent of anthracnose, was detected in samples from Khartoum, River Nile, Sennar, White Nile and Blue Nile States. Different types of fungi were however, isolated from bananas showing symptoms of crown rot. In this respect *F. moniliforme, F. semitectum, F. oxysporum, C. musae*. *V.theobromae* and *Curvularia* sp. were isolated from samples collected from Khartoum.
Table 1: Different fungi isolated from banana peels showing symptoms of different post harvest diseases.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigar end rot</td>
<td><em>Verticillium theobromae.</em></td>
</tr>
<tr>
<td></td>
<td><em>Trachysphera fructigena</em></td>
</tr>
<tr>
<td>Anthracnose</td>
<td><em>Colletotrichum musae,</em></td>
</tr>
<tr>
<td>Crown rot</td>
<td><em>Fusarium semitectum,</em> <em>F. moniliforme,</em></td>
</tr>
<tr>
<td></td>
<td><em>F. oxysporum,</em> <em>Colletotrichum musae,</em></td>
</tr>
<tr>
<td></td>
<td><em>Verticillium theobromae</em></td>
</tr>
<tr>
<td></td>
<td><em>Curvularia sp.</em></td>
</tr>
<tr>
<td>Finger rot</td>
<td><em>Botryodiplodia theobromae</em></td>
</tr>
<tr>
<td>Other rots</td>
<td><em>Cladosporium sp.</em>, <em>Aspergillus niger,</em></td>
</tr>
<tr>
<td></td>
<td><em>A. flavus, A. terreus,</em></td>
</tr>
<tr>
<td></td>
<td><em>Rhizopus stolonifer, Yeast</em></td>
</tr>
</tbody>
</table>
Table 2: Fungi isolated from banana samples showing different disease symptoms.

<table>
<thead>
<tr>
<th>State</th>
<th>Cigar end rot</th>
<th>Anthracnose</th>
<th>Crown rot</th>
<th>Finger rot</th>
<th>Other rots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aspergillus flavus, Aspergillus terreus, Rhizopus stolonifer. Yeasts</td>
</tr>
<tr>
<td>Kassala</td>
<td>Verticillium theobromae</td>
<td></td>
<td>Fusarium semitectum, Fusarium moniliforme, Fusarium oxysporum.</td>
<td></td>
<td>Cladosporium sp. Aspergillus niger Aspergillus flavus. Yeasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aspergillus flavus. Yeasts</td>
</tr>
</tbody>
</table>

(36)
Table 2: Continued

<table>
<thead>
<tr>
<th>State</th>
<th>Cigar end rot</th>
<th>Anthracnose</th>
<th>Crown rot</th>
<th>Finger rot</th>
<th>Other rots</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equatoria State</strong></td>
<td><em>Trachysphera fructigena</em>&lt;br&gt;<em>Verticillium theobroma</em></td>
<td></td>
<td></td>
<td></td>
<td><em>Cladosporium sp.</em>&lt;br&gt;<em>Aspergillus niger</em>&lt;br&gt;<em>Aspergillus flavus, Yeasts,</em>&lt;br&gt;<em>Aspergillus terreus</em></td>
</tr>
<tr>
<td><strong>Sennar State</strong></td>
<td><em>Verticillium theobromae</em></td>
<td><em>Colletotrichum musae.</em></td>
<td><em>Fusarium moniliforme, Fusarium oxysporum, Verticillium theobromae</em></td>
<td><em>Botryodiploia theobromae</em>&lt;br&gt;<em>Fusarium moniliforme</em>&lt;br&gt;<em>Verticillium theobromae</em>&lt;br&gt;<em>Colletotrichum musae</em></td>
<td><em>Cladosporium sp., Rhizopus stolonifer</em></td>
</tr>
<tr>
<td><strong>White Nile State</strong></td>
<td><em>Verticillium theobromae</em></td>
<td><em>Colletotrichum musae.</em></td>
<td><em>Fusarium moniliforme</em>&lt;br&gt;<em>Verticillium theobromae</em>&lt;br&gt;<em>Colletotrichum musae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blue Nile State</strong></td>
<td><em>Verticillium theobromae</em></td>
<td><em>Colletotrichum musae.</em></td>
<td><em>Fusarium moniliforme, Fusarium oxysporum, Verticillium theobromae</em></td>
<td><em>Botryodiploia theobromae</em>&lt;br&gt;<em>Fusarium moniliforme</em>&lt;br&gt;<em>Verticillium theobromae</em>&lt;br&gt;<em>Colletotrichum musae</em></td>
<td><em>Cladosporium sp., Rhizopus stolonifer</em></td>
</tr>
</tbody>
</table>
F. semitectum, F. moniliforme, and F. oxysporum were isolated from Kassala State samples and F.moniliforme and F. oxysporum were isolated from River Nile State samples. *Fusarium semitectum*, Curvularia sp., Fusarium moniliforme, Fusarium oxysporum and *Verticillium theobroma* were isolated from samples from Sennar and Blue Nile States. *Fusarium moniliforme Verticillium theobromae* and *Colletotrichum musae* were isolate from the peel of the samples from White Nile state. However, this disease was not detected in samples from Equatoria State (R.S.S.). Finger rot disease was encountered in samples from Khartoum, Sennar and Blue Nile States and *B. theobromae* was isolated from all the peels of the samples which showed the symptoms of this disease. Other types of rots were recorded in samples from all states. *R. stolonifer, A. niger, A. flavus, A. terreus, yeasts* and *Cladosporium* sp., were isolated from other different types of rots.

Table 3 show the incidence of post harvest diseases calculated on the basis of total healthy and diseased fruits. Cigar end rot was encountered in higher percentages in all states. The percentages were 60%, 50% ,45% and 40%, for the samples from Kassala, River Nile Equatoria(R.S.S) and Khartoum States respectively and 25% for the samples from the remaining states (Sennar, Blue and White Nile). The incidence recorded for crown rot disease was 27%, 20%, 15% and 5% for the samples from Sennar, Khartoum, River Nile and Kassala States respectively and 40% for Blue and White Niles. However, crown rot disease has not been recorded in Equatoria State. The percentage incidence of anthracnose disease was 50, 26, 20, 15 and 10 for the samples from Sennar, Blue and White Niles, River Nile and Khartoum States respectively. The disease has not been recorded in any of the remaining states.
Table 3: Incidence of post harvest diseases in different states in Sudan.

<table>
<thead>
<tr>
<th>State</th>
<th>Incidence (%) of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cigar end rot</td>
</tr>
<tr>
<td>Khartoum</td>
<td>40</td>
</tr>
<tr>
<td>Kassala</td>
<td>60</td>
</tr>
<tr>
<td>River Nile</td>
<td>50</td>
</tr>
<tr>
<td>Equatoria (R.S.S.)</td>
<td>45</td>
</tr>
<tr>
<td>Sennar</td>
<td>25</td>
</tr>
<tr>
<td>Blue Nile</td>
<td>25</td>
</tr>
<tr>
<td>White Nile</td>
<td>25</td>
</tr>
</tbody>
</table>
Finger rot disease was encountered in Khartoum, Sennar and Blue Nile States with incidences of 15%, 6% and 5%, respectively and was not recorded in the other seven states. Other rot diseases were detected in all surveyed states with variable degree of incidence.

**4.2.2. Isolation of fungi in culture media:**

Fungal isolates recovered from the fruits showing cigar end rot symptoms were: *V. theobromae* and *T. fructigena* which is in line with the findings of Snowdon (1990) and Ismail (2004). The isolated fungus from fruits showing symptoms of anthracnose was *C. musae*. Similar findings were reported by Smoot *et al.* (1971), Ismail (2004) and Lapeyre and Mourichon (1997). The fungi isolated from crown rot were *F. semitectum, F. moniliforme, F. oxysporum, C. musae,* and *V. theobromae*. This result agrees with the findings of Mesturino and Ragazzi (1988), Martin *et al.* (1996), Lapeyre and Mourichon (1997), Ogundero (1987) and Ismail (2004). *B. theobromae* was isolated from peels of the samples with finger rot symptoms which confirmed the results by Dadzie and Orchard (1997).

**4.2.3. Single spore isolation:**

The different isolated fungi from mono spore cultures were described and photographed.

**4.2.3.1. Description of fungal isolates:**

*Fusarium oxysporum*

Culture grey to purple; Micro conidia curved, produced from simple short lateral phialides; Macroconidia generally with 3 to 5 septa (Plate 3) Booth (1977).
Plate 3: *Fusarium oxysporum*

a: Culture

b: spores
**Fusarium moniliforme**
Culture violet; Microcoindia 1-septate and produced in chains from lateral phialides (Plate 4).

**Fusarium semitectum**
Culture brown; Macroconidia 3-5 septate (Plate 5).

**Colletotrichum musae**
The acervuli generally dark in colour (Jay, 1986); Conidia aseptate, dark brown (Plate 6).

**Verticillium theobromae** *(Turc)* **Mason & Hughes**
Conidia hyaline; Conidiophore brownish (Plate 7) (Hawksworth and Holiday, 1970).

**Trachysphaera fructigena** **Tabor & Bunting**
Conidia are hyaline; Oogonia thick (Plate 8) Holiday (1970),

**Botryodiplodia theobromae.**
The spores are brown in colour and big (Plate 9). Holiday (1970).
Plates 4: *Fusarium moniliforme*

a: Culture

b: Phialides and spores
Plate 5: *Fusarium semitectum*

a: Culture

b: Spores
Plate 6: *Colletotricum musae*

a: Growth habit

b: Culture

c: Spores

d: Acervulus
Plate 7: *Verticillium theobromae*

a: Conidiophore phialides and spores

b: Culture

c: Growth habit
Plate 8: *Trachysphaera fructigena*

(Oospore)
Plate 9: *Botryodiplodia theobromae*

a: Culture

b: Mature and immature pycnydiospore
4.3. Proof of Pathogenicity according to Koch’s postulates:

The inoculated fruits showed similar symptoms as observed on the diseased fruits when first collected from the market (Plate10 and11). Some of the isolates: *Aspergillus* spp. and *Yeasts* spp. failed to cause infection and were therefore considered as secondary contaminants (Plate 10). Some observations on the development of disease symptoms are listed in the Table 4.

Table 4 results of this experiment proved that isolated *Fusarium* spp., *Verticillium* sp., *Trachysphaera* sp. are pathogenic causing the banana fruit diseases. Other isolated fungi *Aspergillus* spp. and *Yeasts* failed to cause banana fruit infection; and they are considered as secondary contaminants on the banana fruits collected from the market.

*Verticillium* spp. alone or in association with *Trachysphaera* spp., are the causal agent of Cigar-end rot in Sudan.
Plate 10: Secondary infection by *Aspergillus* spp.
Plate 11: Pathogenicity tests

a: Fruit treated with *Fusarium moniliforme*
b: Fruit treated with *Fusarium oxysporum*
c: Fruit treated with *Verticillium theobromae*
d: Fruit treated with *Colletotrichum musae*
e: Fruit treated with *Botryodiplodia theobromae*
f: Control
Table 4: Observed disease symptoms due to inoculation by different Fungi.

<table>
<thead>
<tr>
<th>No</th>
<th>Fungus</th>
<th>Symptoms after 2 days</th>
<th>Symptoms after 6 days</th>
<th>Koch’s postulates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Colletotrichum musae</em></td>
<td>Black necrotic lesion</td>
<td>Sunken brown spots develop with orange acervuli</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td><em>Fusarium moniliforme</em></td>
<td>Dark spots and some white mycelium.</td>
<td>Larger dark spots and the white mycelium become ashy and dry.</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td><em>Fusarium semitectum</em></td>
<td>Dark spots and some white mycelium.</td>
<td>Larger dark spots and some white mycelium.</td>
<td>+ve</td>
</tr>
<tr>
<td>4</td>
<td><em>Fusarium oxysporum</em></td>
<td>Elongated brown spots and clear white mycelium.</td>
<td>Layer elongated dark spots, thick white mycelium.</td>
<td>+ve</td>
</tr>
<tr>
<td>5</td>
<td><em>Verticillium theobromae</em></td>
<td>Elongated brown spots with white mycelium.</td>
<td>The spots become dark, dry and larger, the mycelium turning dry and ashy.</td>
<td>+ve</td>
</tr>
<tr>
<td>6</td>
<td><em>Trachyspher a fructigena</em></td>
<td>Dark spots and with white mycelium.</td>
<td>Larger dark spots and thicker white mycelium.</td>
<td>+ve</td>
</tr>
<tr>
<td>7</td>
<td><em>Botryodipodia theobromae</em></td>
<td>Brownish black discoloration of the peel and a softening of the pulp</td>
<td>The pulp is reduced to a soft (or semi-liquid state), rotten mass, and a dark gray mould grows on the peel surface.</td>
<td>+ve</td>
</tr>
<tr>
<td>8</td>
<td><em>Aspergillus niger</em></td>
<td>Samples observed</td>
<td>Samples observed</td>
<td>-ve</td>
</tr>
<tr>
<td>9</td>
<td><em>Aspergillus terreus</em></td>
<td>Samples observed</td>
<td>Samples observed</td>
<td>-ve</td>
</tr>
<tr>
<td>10</td>
<td><em>Aspergillus flavus</em></td>
<td>Samples observed</td>
<td>Samples observed</td>
<td>-ve</td>
</tr>
<tr>
<td>11</td>
<td>Yeasts</td>
<td>Samples observed</td>
<td>Samples observed</td>
<td>-ve</td>
</tr>
</tbody>
</table>
4.4 Control
4.4.1 *In vitro* biological control:

Effect of *Trichoderma* species on growth of five fungal banana pathogens viz *V. theobromae, F. oxysporum, F. moniliforme, C. musae,* and *B. theobromae* is shown in Table 5. Data recorded 5 days after inoculation showed pronounced inhibitory (antagonistic) effect of *Trichoderma* species against all of tested fungi (Plate 12). Of the three *Trichoderma* spp. tested for their effectiveness against mycelial growth, *T. koningii* and *T. viride* exhibited maximum inhibition against all tested pathogens over the controls with reduction ranging from 39 to 70%. *T. koningii* inhibited *F. moniliforme and F. oxysporum* (53%), *C. musae* (70%) *V. theobromae* (65%) and the least performance were shown with *B. theobromae* where the inhibition was 38%.

*T. viride* suppressed the growth of the five pathogens with inhibition percentages in the range of 65% to 39%. *T. harzianum* showed the least effect and it inhibited the tested pathogens with reduction ranging from 23 to 40%. It should be noted that *Trichoderma* spp. has showed very low inhibitory effect (23 – 39%) against *B. theobromae.*
Table 5: Inhibition shown by *Trichoderma* spp. against different pathogenic fungi.

<table>
<thead>
<tr>
<th>Bio agents</th>
<th>Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Fusarium moniliforme</em></td>
</tr>
<tr>
<td><em>Trichoderma harzianum</em></td>
<td>40</td>
</tr>
<tr>
<td><em>Trichoderma koningii</em></td>
<td>53</td>
</tr>
<tr>
<td><em>Trichoderma viride</em></td>
<td>56</td>
</tr>
</tbody>
</table>
4.5. Control of post harvest diseases of banana fruits using plant extracts:

The plant extracts of neem and garlic inhibited mycelial growth of *V. theobromae, F. moniliforme, F. oxysporum, C. musae* and *B. theobromae* by different efficacy. Neem extracts had the highest inhibitory effect against *C. musae* and recorded 60% and 48% for cold and hot water extracts, respectively. Garlic extracts had the highest inhibitory effect against *C. musae* recording 59% and 41% in cold and hot water extracts, respectively.

Both neem and garlic extracts had low effect against *B. theobromae* with inhibitory percentages ranging between 7-13% in cold and hot water extracts. The efficacy of cold water extracts was consistently higher than hot water extracts. In comparison, Byfidan fungicide inhibited the growth of all tested pathogens, except for *B. theobromae* (Fig. 1).
a: *Fusarium moniliforme*

b: *Fusarium oxysporum*

c: *Verticillium theobromae*
Plate 12: *In vitro* antagonistic effect of *Trichoderma* spp. on different banana pathogens

**T₁:** *Trichoderma viride*

**T₂:** *Trichoderma harzianum*

**T₃:** *Trichoderma koningii*

*d: Botryodiplodia theobromae*

e:*Colletotrichum musae*
Figure 1: Effect of neem and garlic aqueous extracts on banana pathogenic fungi compared with bayfidan fungicide
Fungal isolates from the fruits showing cigar end rot symptoms were *V. theobromae* and *T. fructigena* and these results go with the findings of Snowdon (1990). The isolated fungus from fruits showing symptoms of anthracnose was *C. musae* the result in agreement with Smoot *et al.* (1971); Bilgrami *et al.* (1979, 1981) and Lapeyre and Mourichon (1997). The fungi isolated from crown rot were *F. semitectum*, *F. moniliforme*, *F. oxysporum*, *C. musae*, and *V. theobromae* which is in agreement with the findings of Mesturino and Ragazzi (1988), Martin *et al.* (1996), Lapeyre and Mourichon (1997), and Ogundero (1987). Similarly, the results of isolating *B. theobromae* from peels of samples with finger rot symptoms are in line with the results of Dadzie and Orchard (1997).

From the results shown in Table 2 it appears that the isolated pathogenic fungi that considered as the causal agents of different fungal post harvest diseases of banana differ from one state to the other and this may be due to variation in environmental conditions such as temperature and humidity etc... that favors their growth.

The rate of occurrence of cigar end rot was the highest in all states. The wide occurrence of cigar end rot disease may be due to that good field practices, such as pulling of the flowers remains from the fruits is not followed by the farmers in Sudan.

The effects of the antagonists against banana pathogens indicated that
*T. harzianum, T. koningii* and *T. viride* in the present study showed that all the three antagonists performed well but with varying levels of specificity. Bhuvaneswari and Rao (2001) evaluated postharvest pathogens of mango. They found that the growth of *Pestalotia* sp., *A. flavus, Lasiodiplodia theobromae, Colletotrichum gloeosporioides,* *R. stolonifer* and *A. niger* was inhibited in varying degrees by antagonistic fungi.

The results of use of cold and hot water extracts of neem and garlic plants for *in vitro* control of the tested fungi go in line with the findings of Bankole (1994), Obagwu *et al.* (1997), Adetogun and Atayese (2006), Eziashi *et al.* (2006), Muhamad and Mustapha (2006), Akpa and Amodu (2006), Ogbebor *et al.* (2007) and Nduagu *et al.* (2007) who reported that plant extracts have great potentialities to control different pathogenic fungi. Zarafi and Moumoudou (2010) used both cold and hot water extracts of neem and garlic against *Curvularia eragrostidis* the causal agent of mid rib spots of pearl millet and they found that these extracts reduce the mycelial growth of the fungus.

Cold water plant extracts inhibited the growth of pathogens more than hot water extracts, this may be due to that the active components of these plant extracts are broken down by heat. Lubna and Husham (2007) reported that exposure of neem extracts to high temperatures (55°C and
above) caused most of the components to disappear or their concentration significantly decreased.

Fungi causing banana fruit diseases are wide spread, air-borne, host-borne, soil-borne and are abundant in any banana plantation. Most of the organisms become infective and destructive when the fruits are injured or bruised due to improper handling, insect damage or poor plantation husbandry. Injured fruits become readily susceptible to infection. Efforts to better, pre-harvest and post- harvest practices should be made in order to minimize the possibilities of infection, especially by air-borne fungi. Post-harvest and storage practices of fruits in the Sudan are primitive, causing physical damage such as scratches and bruises of fruits. This coupled with non refrigerated storage, cause great physical and pathological damage, and increase fungal losses (Silvis, 1974). During non- refrigerated lorry transport in Sudan, the banana fruits are covered with collected dead banana plants leaves, usually carrying a very high spore’s inoculums of infective fungi. The high humidity conditions created within the cargo by the leaves, and fruit respiration with high temperatures and long journey distances, from Damazin and Kassala, help in creating optimal conditions for the growth of fungi, including those, which will initiate infection of the damaged fruits.

Marketing arrangements in the Sudan are also primitive. Banana fruits in the market place are not refrigerated and are exposed open, to air-borne
microorganisms, insect and dust, at warm to high temperatures, encouraging infections of banana hands, crowns and fruits, and thus shorten their shelf-life; since the fruits and crowns are not protected from dirt and dust, which fall on physically exposed crowns and bruised fruits, causing crown rot disease (Ismail, 2004).
RECOMMENDATIONS

Sudan has a high potential for producing banana for local market and export, but is still needs for good organizational practices including pre-harvest plantation methods and proper post-harvest handling and marketing techniques. Much work is needed to improve production and marketing of bananas inside the Sudan.

There is an urgent need to find alternative control methods for postharvest diseases that do not rely on fungicide chemicals, since pathogens build up resistance to these chemicals, and since there are increasing concerns about the effect of pesticides on human health. Future studies will therefore be aimed at developing suitable formulations for antagonist mixtures to be used on a large scale in postharvest conditions.
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Society for Plant Protection (NSPP) held at Nasarawa State University, Keffi.


