IDENTIFICATION AND MANAGEMENT OF FUNGI ASSOCIATED WITH CROWN ROT OF BANANA IN MAKURDI, BENUE STATE, NIGERIA

Ekhuemelo*, C. and Yaaju, M. D.

ABSTRACT

*Department of Crop and Environmental Protection, Federal University of Agriculture, P.M.B. 2373 Makurdi, Benue State, Nigeria. * Corresponding author. Email: chiekhuemelo@gmail.com. Tel: +234 (0)7031332667*

The study was undertaken to determine the fungi responsible for the post-harvest rot of banana fruit in Makurdi and management of the prevalent fungi using plant extract. Pathogenicity test revealed that fruit rot was caused essentially by Botryodiplodia theobromae, Aspergillus parasiticus and Aspergillus niger on healthy banana fruits with B. theobromae being the most virulent. The in-vitro experiment consisted of potato dextrose agar media amended with the aqueous extracts of Ginger (Zingiber officinale) and Garlic (Allium sativum) at three concentrations of 10%, 20% and 30% and inoculated with Botryodiplodia theobromae and an untreated control. The growth inhibition trends indicated that 30% garlic > 20% garlic > 30% ginger> 20% ginger > 10% garlic > 10% ginger > control (no extract) at 3, 4, 5 and 6 days after inoculation (DAI). The fungitoxic effect of the extracts of garlic induced mycelia inhibitions ranging from 15.5 - 100% while ginger extract induced mycelia inhibitions ranging from 3.2- 57.6% at 3 DAI. This study demonstrated that the use of garlic extract at 30% w/v in-vitro was able to effectively reduced the mycelia growth of B. theobromae causal agent of banana crown rot in Makurdi.

Keywords*: Pathogenicity, Allium sativum, Zingiber officinale, inhibition*

INTRODUCTION

Banana (*Musa acuminata* Colla) is an edible fruit (botanically a berry) of the genus Musa (Vezina, 2016). Bananas are grown throughout the tropics. Nigeria is a major producer of banana in West Africa (Cauthen *et al*., 2013). In Nigeria 450,000 hectares are utilized for banana production with 2.8 metric tonnes produced in 2013 (Vezina, 2016). Benue State is known for banana production in Nigeria (Akinyemi *et al*., 2010). Bananas are a rich source of natural sugars such as glucose, fructose and sucrose, vitamin B6**,** vitamin C**,** potassium**,** calcium, phosphorus, dietary fibre**,** biotin**,** carbohydrates, magnesium**,** riboflavin**,** manganese, and dietary fiber (Cauthen *et al*., 2013; Yakub, 2015). Genotype and preparation method influences the nutritional content of banana (Cauthen *et al.*, 2013).

Banana is a staple crop that can be eaten raw, deep fried, pounded and eaten with soup, baked in the skin or steamed in glutinous rice wrapped in banana leaf or made into jam. Dried bananas are also ground to make banana flour. Banana powder is used as the first baby food (Cauthen *et al*., 2013; Yakub, 2015). Banana pancakes are popular amongst the people of South Asia and South East Asia. Banana can be mixed with other fruits in fruit salad, sliced on cereal or yogurt, combined in a sandwich with peanut butter (Cauthen *et al*., 2013). They are also used in making banana beer, alcoholic beverages, vinegar, as ornamental plants, as cattle feed and for shade (Yakub, 2015). Banana leaves are often used as disposable food containers or as "plates" in South Asia and several Southeast Asian countries and West Africa. Banana leaf is used as packages when cooking some food (Cauthen *et al*., 2013; Yakub, 2015). Dried banana peels are used in making soap. The dried leaves and sheath are used as sponges and as roofing material (Akinyemi *et al*., 2010).

Postharvest losses of banana due to postharvest rot diseases reduce the quality and shelf life of banana fruits and results in substantial losses to farmers and retailers. Banana is a climacteric fruit prone to postharvest losses due to its high respiratory rate and ethylene production during storage and marketing (Turner, 2001). Postharvest losses of 30- 40 % have been reported in Nigeria with 30% occurring at the wholesale and 70% during retailing (Olorunda, 1996). The prevention and management of postharvest diseases have been largely neglected. Previous studies have been focused on varietal improvement and prevention of field diseases. The use of non-chemical alternatives which are relatively cheap and environmentally friendly in the management of post-harvest diseases of banana will prevent the development of such diseases and increase revenue of banana farmers. The study was conducted to identify the fungi associated with banana fruit rot in Makurdi and to determine the effect of aqueous hot water extracts of *Zingiber officinale* and *Allium sativum* in the management of fungi associated with crown rot of banana fruit.

MATERIALS AND METHODS

Pathogen isolation

Infected banana fruits with symptoms of pulp and crown rot collected from Wurukum, North bank and Modern markets in Makurdi Local Government Area of Benue State were used for isolation of fungal organisms associated with banana fruits. Longitudinal sections of 3-5mm^2 were cut from the edge of the infected lesions

(Agrios, 2005) and sterilized for one minute in 10% Sodium hypochlorite solution and rinsed in three changes of Sterile Distilled Water (SDW). The sterile pieces were blotted dry on sterile filter papers and placed on Potato Dextrose Agar (PDA) in 9cm sterile Petri dishes. The dishes were incubated at ambient conditions of light and temperature (30± 2 °C) for 3 days after which pure cultures were obtained by sub culturing unto fresh PDA plates. Pure cultures were identified using compound microscope and compared with reference manual (Wanatabe, 2010) and further confirmed at the Germplasm Health laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Pathogenicity tests

Pathogenicity test was carried out on the fungi isolated from banana fruits to confirm the pathogenic organisms. The pathogenicity of three isolates namely: *Botryodiplodia theobromae, Aspergillus niger and Aspergillus parasiticus* was tested *in vivo* on detached unripe healthy banana fruits using agar plug method of inoculation (Ekhuemelo *et al*., 2016). Thirty six apparently healthy banana fruits were surface sterilized with 10% Sodium hypochlorite for one minute and washed in three changes of sterile distilled water. A 3mm cork borer was used to punch into the healthy banana fruits and the bored tissues were removed. A 1mm cork borer from pure cultures of the three fungi was used to inoculate the banana fruits (three banana fruits were inoculated per replicate of each test fungus) and the tissue replaced. Lesion diameter was measured based on the symptoms induced at three days after inoculation at ambient conditions of light and temperature using a metre rule.

Severity rating was recorded using a modified severity key adopted from Enikuomehin (2005): 0= no infection (no spot/no infection), 1= 1- 20 % of fruits with small lesions, 2 = 21- 40 % of fruits with lesions, 3= 41- 60 % of fruits with lesions, 4 = 61- 80 % of fruits with lesions and 5 = 81- 100 % of fruits with lesions.

The organisms were re-isolated from inoculated fruits and confirmed with the original fungi introduced.

Management of postharvest rot on banana

Preparation of plant extract

Crude extracts of Ginger (*Z. officinale*) and garlic (*A. sativum*) were used for the experiment. The fresh plant materials were purchased from North bank market, peeled, rinsed with distilled water, pounded using a mortar and pestle, filtered with double layer cheese cloth. The concentrations were obtained by weighing 10g, 20g and 30g extract in 100ml distilled water in 250ml Conical flask to give 10%, 20% and 30% concentration of each plant extract. Each concentration of the plant extract was boiled separately at 60^{o} C for 20minutes. Control was Sterile Distilled Water.

Media amendment with plant extracts

Four grams of PDA (Lab M) was added to each concentration of plant extracts and the flasks were autoclaved at 121^{o}C for 15minutes (Nduagu *et al*., 2008). After autoclaving each medium streptomycin sulphate (100mg per litre) was added to prevent bacterial contamination. Media were allowed to cool to about 40^{o}C and poured into sterile 9.0cm Petri dishes (Obagwu *et al*., 1997).

Fungitoxic effect of plant extracts

The media amended with plant extracts were inoculated at the centre with mycelia discs (1mm diameter) taken from the advancing edges of 5 days-old pure culture of *Botryodiplodia theobromae*. The experiment was a 2x 4 factorial experiment (consisting of two plant extracts Ginger (*Z. officinale*), Garlic (*A. sativum*) and three concentrations (10%, 20% and 30%) and a control (where no extract was added) laid out in a completely randomized design replicated three times. The inoculated media were incubated at ambient conditions of light and temperature (30±2^{o}C). The diameter of the fungal colony was measured using a meter rule along two diagonal lines drawn on the reverse side of each Petri dish from 3 days after incubation.

The percentage reduction (Mr) of mycelia growth by each extract was computed using the formula (Nduagu *et al*., 2008):

$$Mr = \frac{M1 - M2}{M1\ x\ 100}$$ ---------- Equation 1

Where M_r = % reduction in mycelia growth, M_1 = mycelia growth in the untreated medium (control); M_2 = mycelia growth on the treated medium.

The percentage inhibition was rated using the method of Okigbo *et al*. (2009) where;

≤ 0 % inhibition (Not effective), > 0- 20 % inhibition (Slightly effective), > 20-50 % inhibition (Moderately effective), > 50- < 100 % inhibition (Effective) , 100 % inhibition (Highly effective).

Data analysis

The experiment was a 2 x 4 factorial experiment laid out in completely randomized design. Data were subjected to analysis of variance (ANOVA) using SAS version 9.2 statistical software package (SAS, 2009) and significantly different treatment means compared with Fishers Least Significant Difference (FSLD) at 5% level of probability (Obi, 2002).

RESULTS

Morphological and microscopic description of *Botryodiplodia theobromae* is presented in Fig. 1. The mycelium was hyaline and well branched. Main hyphae were found to be up to 6–8 µm wide. Average radial growth of the

oomycete at 25°C on PDA is 11 mm per day. Sporangia are globose to somewhat cylindrical, measuring 15–55 µm in diameter and 65 µm in length.

Figure 2 shows the morphological and microscopic description of *Aspergillus niger*. Colonies on PDA at 27 °C attained a diameter of 4-5 cm within 7 days consisting of a compact white or yellow basal felt with a dense layer of dark brown to black conidiophores. Conidial heads, black, radiate, tending to split into columns with age. Conidiophores are stipes smooth walled hyaline and in brown colour. Phialides borne on metulae, 7.0-9.5 x 3-5µm, metulae hyaline to brown, septae, 15-25 x 5.0 -6.0 µm. Conidia globose to subglobose. Morphological and microscopic description of *Aspergillus parasiticus* is presented in Fig. 3. Colonies dark green surface pigmentation with a suede-like surface consisting of a dense felt of conidiophores. Conidial heads green, radiate, conidiophores mostly 300-700µm long, hyaline, rough-walled. Vesicles subglobose, 20-35µm in diameter, Phialides borne directly on the vesicle, 7-9 x 3-4µm, hyaline to pale green.

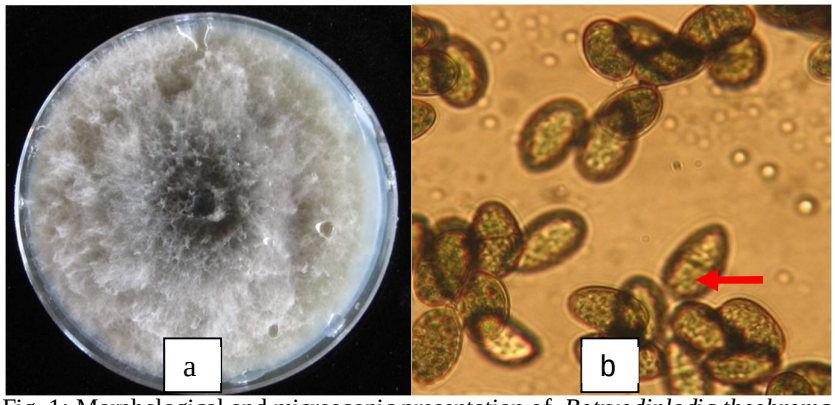

Fig. 1**:** Morphological and microscopic presentation of *Botryodiplodia theobromae* showing (a) **W**ell branched mycelia growth **(**b) Oval spores

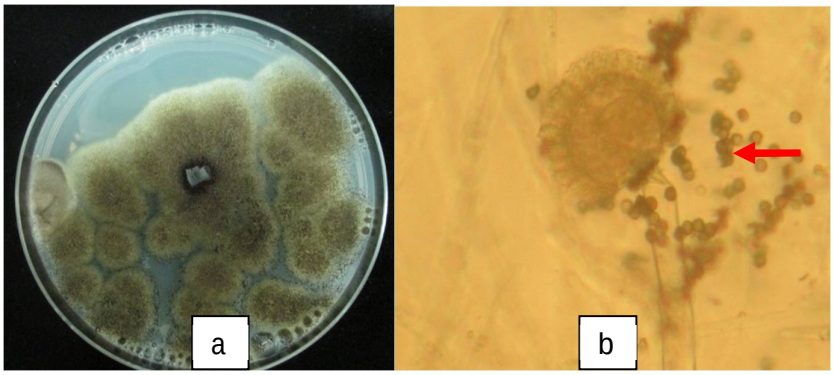

Fig. 2: Morphological and microscopic presentation of *Aspergillus niger* showing (a) Black **c**olony growth (b) spores and vesicle

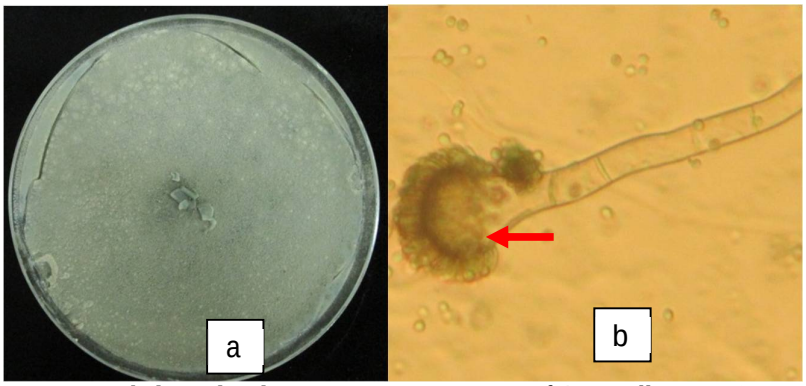

Fig. 3: Morphological and microscopic presentation of *Aspergillus parasiticus* showing (a) Dark green colony growth with a suede-like surface (b) subglobose vesicle.

Pathogenicity and severity rating for fungal organisms associated with banana (*Musa acuminata* Colla) fruit in Makurdi, Nigeria is shown in Table 1. Three different fungi namely *Botryodiplodia theobromae* Pat, *Aspergillus parasiticus and Aspergillus niger* Van Tiegh were isolated from the banana fruits in Makurdi, Nigeria. All the three isolated fungi specie were pathogenic as they caused rot in healthy banana fruits. *B. theobromae* was found to be the most virulent on banana fruits with a severity rating of 4.33.

The pathogenicity test shows that *Botryodiplodia theobromae* had significantly ($p<0.05$) higher lesion diameter (16.71 cm) followed by *Aspergillus niger* (6.44 cm) and *Aspergillus parasiticus* (1.46 cm) at 3 days after inoculation (DAI). Growth rate of *B. theobromae* was rapid and covered the 9.00 cm Petri dish as from 3 DAI. The severity ratings show that *Botryodiplodia theobromae*>*Aspergillus niger* >*Aspergillus parasiticus*.

Concentration effect of plant extracts

Table 2 and Fig. 1 show the effect of different concentrations of hot water extracts from ginger and garlic on mycelia growth of fungal organisms associated with banana (*M. acuminata* Colla.) fruits in Makurdi, Nigeria. *Allium sativum* extract at 30% concentration completely suppressed and inhibited the mycelia growth of *B. theobromae* by 100% 3 DAI and was classified as highly effective. *Zingiber officinale* extracts at 10% recorded the lowest inhibition of mycelia growth of 3.20% (slightly effective) at 4 DAI. The growth inhibition trends show that 30% garlic > 20% garlic > 30% ginger> 20% ginger > 10% garlic > 10% ginger > control (no extract) at 3, 4, 5 and 6 DAI.

Table 1: Pathogenicity and severity rating of isolated fungi

Treatment	Lesion diameter	Severity rating
Aspergillus parasiticus	1.46 ± 0.28	1.00 ± 0.00
Botryodiplodia theobromae	16.71 ± 0.34	4.33 ± 0.33
Aspergillus niger	6.44 ± 0.75	2.67 ± 0.33
F-LSD$_{(0.05)}$	1.73	0.94

Table 2: Effect of three concentrations of plant extracts on the mycelia growth of *Botryodiplodia theobromae* isolated from banana fruits in Makurdi, Nigeria

Plant extract	Mycelia growth (cm)			
	3DAI	4DAI	5DAI	6DAI
Zingiber officinale				
10	8.23	9.00	9.00	9.00
20	4.73	7.65	8.07	9.00
30	3.60	6.67	7.50	8.33
Allium sativum				
10	7.18	9.00	9.00	9.00
20	4.67	5.33	6.17	6.67
30	0.00	0.00	0.00	0.00
Control	8.50	9.00	9.00	9.00
FLSD	0.71	0.52	0.31	0.52

DAI= days after inoculation

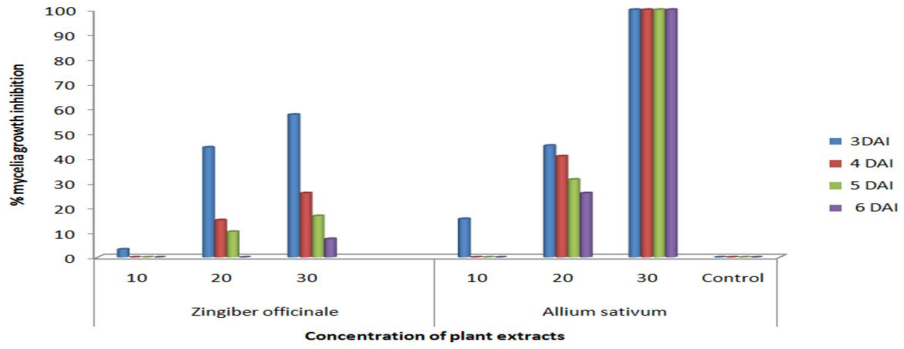

Fig. 1: Effect of plant extract concentrations on the percentage inhibition of the mycelia growth of *Botryodiplodia theobromae.*

DISCUSSION

Fungal organisms associated with the postharvest rot of banana in this study were *Botryodiplodia theobromae, Aspergillus niger and A. parasiticus.* These pathogens were isolated from rotted banana fruits and their ability to infect banana fruits were confirmed through pathogenicity tests. The pathogenicity tests revealed that all the three

fungi induced rot in banana fruits with *Botryodiplodia theobromae* being the most pathogenic with a growth of 11 mm per day leading to rotting of inoculated fruits in 3 days. Although Diedhiou *et al*. (2014) attributed infections on banana fruits to the presence of *Colletotrichum musae* and *Fusarium* spp. alone or in association with other fungi, such as *Aspergillus flavus and Aspergillus niger, B. theobromae* was found in association with *A. niger* and *A. parasiticus* in this study.

Crown rot of banana caused by *B. theobromae* is an important postharvest disease of banana fruits. The disease has been reported in more than 70% of farms in Nigeria and is known to cause losses of about 80% of crop harvest (Onyenka *et al*., 2005). Nelson (2008) and Yakub (2015) had earlier identified *B. theobromae* as one of the fungi responsible for crown rot in banana. Also Okigbo *et al.* (2013) and Twumasi *et al*. (2014) reported *B. theobromae* as the causal agent of rot in banana and yam. The morphological description of *B. theobromae* in this study is in line with the general description of *B. theobromae* as reported by Shah *et al*. (2010) and Twumasi *et al*. (2014). Triest and Hendrickx (2016) observed that crown rot infection begins at harvest with the mycelium developing on dead banana stalks or leaf, spreading unto the crown surface during the cutting of the bunch resulting in softening and blackening of the fruit tissue. Furthermore, Ayinde *et al.* (2010) reported that banana consumers preferred the absence of black spots caused by *B. theobromae* on banana peel. The use of garlic extract at 30% w/v will be able to control the mycelia growth of *B. theobromae* thereby preventing the formation of black spots on banana fruits and increasing the preference of such fruits by consumers.

The higher level of inhibition exhibited with higher concentrations of Garlic and Ginger reveals the importance of concentration levels in extracts effectiveness. This is in line with the report of Ojo and Olufolaji (2011) that the inhibitory action of different extracts on mycelia growth increased with increasing concentration. Also the reduction of mycelia growth by *Allium sativum* in this study agrees with the report of Ojo and Olufolaji (2011) in which aqueous extracts of *Allium sativum* significantly inhibited the fungal growth of *Colletotrichum gloeosporiodes* by 100 %. A survey conducted to determine the fungicidal properties of ginger rhizome extract reported growth inhibition on *Fusarium*, *Colletotrichum* and *Curvularia* species by ginger rhizome extract by 70.0 %, 71.0% and 64.2 % respectively (Gyasi, 2014). Also a research carried out to test the potency of some plant extracts for the control of yam tuber rot caused by *Fusarium oxysporum* Schlecht, *Aspergillus niger* Tiegh and *A. flavus* Link reported that the hot water extract obtained from ginger (*Zingiber officinale* Rosc.) was fungitoxic against the yam rot fungi and suppressed the growth of *Fusarium oxysporum*, *Aspergillus niger* and *A. flavus* (Okigbo and Nmeka, 2005). In a report by Khan and Kumar (1992) garlic, ginger and neem extract were effective against *Curvularia lunata, Fusarium spp.* on wheat seed. The fungitoxicity of ginger could be attributed to antioxidants such as gingerols and polyphenol which are effective against many diseases that affect cultivated crops (Opara and Obani, 2009).

CONCLUSION

The fungi associated with the postharvest rot of banana fruits were *B. theobromae A. niger and A. parasiticus* with *B. theobromae* being the most virulent. The application of garlic extracts at 30% concentration recorded 100% inhibition of the mycelia growth of *B. theobromae* in-vitro 7 DAI.

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