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Control of Post Harvest Fungal Deterioration of Pineapple (*Ananas comosus* (L) Merr.) Using Extracts of Plant Origin in Nigeria

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Abstract

Post-harvest fungal deterioration of pineapple is the most important challenge in pineapple production value chain causing losses in both nutrient and market values. This research was undertaken to identify the fungal pathogens causing rot of pineapple fruits in storage and their control using the water and ethanol leaf extracts of *Moringa oleifera*, *Azadirachta indica* (Neem), and *Carica payaya* (pawpaw). Pathogenicity test of fungal isolates from infected pineapple fruit indicated *Rhizopus stolonifer, Aspergillus niger* and *Fusarium solani* as major pathogenic organisms causing storage rot of pineapple fruits. *A. niger* had the highest frequency of occurrence and most virulent recording the highest disease incidence and severity followed by *R. solonfer* and *F. solani*. The effects of both aqueous and ethanol extracts of the plant materials on rot development and spread in pineapple fruits by the pathogens showed that pineapple fruits sprayed with the plant extracts before inoculation of the pathogens recorded less disease incidence than those fruits spray-inoculated with pathogen before application of plant extracts suggesting that the extracts are better used as protectant before infection and development of disease. The ethanol leaf extracts were more effective than water extracts in reducing the disease incidence and severity of the pathogenic organisms in pineapple indicating that the solvent of extraction affected the antimicrobial activities of the test plant materials with ethanol extracting more active compounds than water as extracting solvent. The extracts of *A. indica* were most potent in the reduction of rot development and spread in pineapple indicating that the extracts contained more anti-fungal properties that affected the growth of the pathogenic organisms *in vivo* followed by extracts of *C. papaya* and *M. oleifera*. Extracts of these plant materials could therefore be exploited as pesticides of plant origin in the control of postharvest microbial deterioration of pineapple fruits and increase food production.

Keywords: Postharvest, Pathogens, Deterioration, Pineapple, Plant Extracts*, in vivo*

INTRODUCTION

Pineapple (*Ananas comosus* (L) Merr.) is the most economically important plant in the order, Bromeliales, family Bromeliaceae, genus *Ananas,* and species *comosus* (Bartholomew *et al.,* 2003). It is grown in over 82 countries, on over 2.1 million acres, with a global production of 15,287MT and the second most traded product after bananas in tropical countries of the world (FAO, 2009). The top producers are Thailand, Philippines, Brazil, China, India, Costa Rica, Nigeria, Kenya, Mexico, and Indonesia. According to FAOSTAT (2011). Nigeria is the seventh largest producer in the world and the leading producer in Africa, with a production capacity of 1,400,000 metric tons of fresh pineapple. The estimated area under cultivation is 121 thousand hectares, with an average production of 917 thousand tons and a productivity of 7.6 tons/ha, thereby contributing about 13.5% ha of land cultivated and 5.1% tons of pineapple output in the world (Mark, 2010). Pineapple production has gained popularity because of its potential for rural development through increased youth employment, household incomes, poverty reduction and foreign exchange earnings (Ssemwanga, 2007). The major chemical composition or nutrient values per 100g flesh pineapple fruit include; 87 g water, 190 kj energy, 11.82g carbohydrate, 8.29 g sugars, 1.4 g dietary fibre, 0.13 g fat, 0.55 g protein, 4.59 g sucrose, 1-76 g glucose, 1-94 g fructose, 13.0 mg calcium, 31 μg betacarotene, 0.078 mg thiamin (B_1), 0.029 mg riboflavin (B_2), 0.470 mg niacin, 0.193 mg pantothenic acid (B_5), 0.106 mg pyridoxine (B_6), 1 mg sodium, 11 μg, 16.9 mg vitamin c, 0.25 mg iron, 9 mg phosphorus, 11.5 mg potassium, 0.08 mg zinc, 12 mg magnesium and 0.7 μg vitamin K (USDA, 2009). It contains 15% sugar, malic and citric acids as well as vitamins B_1, B_2, B6, and C and Bromelain, a protein digesting enzyme which aid digestion at the end of a high protein meal (Nwosu, 2011; Cheng and Paul, 2011).

A number of constraints could affect production of pineapple, prominent amongst them are pests and diseases. Pineapple fruits are vulnerable to pathogenic attack because of their high moisture content and nutrient composition, which causes them to decay and become unfit for human consumption due to mycotoxin generation (Paull and Chen, 2003). Akinmusire (2011) reported that *Aspergilus flavus, Rhizopus spp. Fusarium spp,* and *Phytopthora spp*. were the major cause of losses of *Ananas comosus* in storage. Diseases such as *Fusarium* stem rot, and a host of other diseases of pineapple have been reported by Evans *et al.* (2002) that cause 20-25% losses of the harvested fruits along the post-harvest chain (Eni *et al.,* 2010). Fungi have been shown to enter host tissues via natural openings such as lenticels and intact epidermis via appresorium or germ tube (Rhoda, 2008).

The use of synthetic chemicals has been suggested in the control of storage rot of pineapple which not only increase production cost but hazardous to the health of farmer and consumer (Duane, and Bartholomew 2009). These problems have necessitated the search for alternative pesticides that are effective and environmentally friendlier than synthetic pesticides. Plant extracts, as biopesticides, are inherently less harmful than synthetic chemicals and readily available, cost effective and biodegradable, thereby resulting in lower persistence and without the pollution problems associated with synthetic chemicals (Mitchell and Kannwischer-Mitchell, 2004). The isolation and identification of fungal pathogens associated with pineapple fruit rot in storage and evaluation of the effects of water and ethanol leaf extracts of *Moringa oleifera*, *Azadirachta indica* (Neem), and *Carica papaya* (paw-paw) against rot development and spread in pineapple (*in vivo*) by rot causing organisms are presented in this paper.

MATERIALS AND METHODS

Experimental Site and Source of Materials

The experiment was carried out in the Plant Health Management Laboratory of Michael Okpara University of Agriculture, Umudike, Umuahia Abia State. The healthy (uninfected) and diseased pineapple fruits were sourced from Umuahia, Abia state. The leaves of *Moringa oleifera, Azadirachta indica* (neem), and *Carica papaya* (pawpaw) were collected from the university community.

Culture Medium

The culture medium was prepared by adding 39g of Potato Dextrose Agar (PDA) to one liter of sterile distilled water in 1000 ml conical flask, shaken and covered with cotton wool wrapped with aluminum foil and then sterilized in an autoclave at 121^0C for 15minutes, allowed to cool before dispensing (15 ml) into sterile Petri dishes.

Isolation and Identification of Pathogens

Diseased pineapple fruit was washed with water and surface sterilized with 70% ethanol and cut into pieces (3mm) and then inoculated on the solidified PDA culture medium in plates. The inoculated plates were incubated at room temperature ($27+2^oC$) and examined daily for fungal growth which were sub-cultured on a fresh PDA medium to obtained pure cultures of the fungi isolates (Amadioha, 2001). Pathogenicity test was carries out on the isolates to confirm pathogenic organisms (Barnett and Hunter, 2006).

Plant Extract

Fresh leaves of *M. oleifera, A. indica* and *C. papaya*, were washed, air-dried and ground to form a paste. Water or ethanol extract of the plant materials was obtained by infusing 100 g each of the leaf paste separately in 100 ml of sterile distilled water or ethanol as solvent to obtain 100% concentration of water or ethanol leaf extracts of the plant materials. The mixture was stirred and left to stand for 2 hours to allow for extraction of the active ingredients and then filtered using four-fold cheese cloth (Wokocha and Okereke, 2005).

Effect of Plant Extract on Rot Development and Spread

Ten surface sterilized healthy (uninfected) pineapple fruits were treated as group A and B.

(A) Set of ten surface sterilized uninfected pineapple was each sprayed with different plant extract and left for two hours before spray-inoculating each separately with spore suspension (1 x 10^5 spores/sterile distilled water) of fungal pathogens. The control experiment was sprayed with distilled water or ethanol alone in place of plant extracts.

(B) A set of ten surface sterilized uninfected fruits were spray-inoculated separately with spore suspension of the fungal pathogens before spraying the plant extracts after 2 hours. For the control experiment, the inoculated fruits were sprayed with distilled water or ethanol alone instead of the water or ethanol extracts of the test plants.

The treated pineapple fruits including the control experiment was each placed in a micro humidity chamber at room temperature and observed daily for rot development. The disease incidence and severity were assessed (Amadioha and Markson, 2007).

$$\text{Disease incidence } (\%) = \frac{\text{Number of rotted fruits}}{\text{Total number of fruits}} \times \frac{100}{1}$$

Disease severity was assessed on a 0 –5 scale as follows:

0. - No infection
1. 1-20% - slight infection
2. 21-40% - moderate infection
3. 41 – 60%. Highly infected
4. 61 – 80% - Extensive infection
5. 81 – 100% - Complete rot

The fungitoxicity of the extracts was determined as a percentage decay reduction in inoculated pineapple (Amadioha, 2003).

Data Analysis

The experimental design was Complete Randomized Design (CRD) with three replicates. The data obtained were subjected to Analysis of variance (ANOVA) using SAS and the mean separated using Least Significant difference (LSD) at 5% level of probability.

RESULTS

Pathogenic Organisms

Table 1 shows frequency of occurrence, disease incidence and severity of infected pineapple fruits sampled. *Aspergillus niger* was detected in all the samples (100%) followed by *Rhizopus stolonifer* (80%) and *Fusarium solani* (60%). The pathogenic organisms showed variation in the extent or degree of rot development and spread in pineapple during pathogenicity test (Plates 1, 2, 3).

Table 1:Frequency of occurrence and rot development and spread in pineapple by pathogenic organisms.

Fungal Pathogin	Frequency of occurrence (%)	Disease Incidence (%)	Severity
Rhizopus stolonifer	80.0	66	4
Aspergillus niger	100.0	81	5
Fusarium solani	60.0	50	3

Plate 1: Pineapple fruit inoculated with *Aspergillus niger*

Plate 2: Pineapple Fruit inoculated with *Rhizopus stolonifer*

Plate 3: Pineapple fruit inoculated with *Fusarium solani*

Effect of plant extract on rot development and spread in pineapple

There were variations in rot development and spread in the inoculated pineapple fruits treated with extracts of the plant materials (Table 2). Complete rot (100%) was recorded in the control experiment treated with water alone both before and after inoculation of the three pathogenic organisms whereas fruits sprayed with ethanol alone before inoculating the pathogens slightly reduced rot development caused by *R. stolonifer, A. niger* and *F. solani* with disease incidence of 85%, 88% and 95% respectively.

The water and ethanol extracts of *M. oleifera, A. indica* and *C. papaya* reduced to varying degrees rot development and spread in pineapple especially when applied before inoculation of the pathogens. The aqueous leaf extract of the plant materials was not as effective as the ethanol extracts in reducing the rot development and spread in inoculated pineapple fruits. Also, extracts of the test plants applied before spray inoculating the pathogens recorded lower disease incidence than when applied after spray inoculating the pathogen. *A. indica* extracts were more effective in reducing the rot development and spread caused by *R. stolonifer* whereas *C. papaya* extracts were more potent in reducing the rot disease incidence incited by *A. niger* and *F. solani.* Ethanol leaf extracts of *A. indica* was more effective than other plant extracts and comparable to the synthetic fungicide (mancozade) in reducing the disease incidence in pineapple inoculated with the test fungi. Both water and ethanol leaf extracts of the plant materials were more effective when compared with the control experiment in the control of rot development and spread in pineapple caused by *R. stolonifer* followed by *A. niger* except *A. indica* and then *F. solani* which recorded the highest disease incidence.

Table 2: Effect of plant extracts on rot development in pineapple caused by pathogens

Treatment	**Pathogens and Disease incidence (%)**					
	Rhizopus stolonifera		***Aspergillus niger.***		***Fusarium solani***	
	A.	**B**	**A.**	**B**	**A.**	**B**
Moringa Olieferia						
Water extract	55.00^{c}	60.00^{e}	70.00^{e}	75.50^{d}	70.00^{d}	80.00^{c}
Ethanol extract	35.03^{b}	50.00^{c}	60.00^{d}	65.00^{c}	70.00^{d}	75.50^{b}
Azadirachta indica						
Water Extract	45.00^{c}	55.00^{d}	75.00^{d}	80.00^{e}	65.00^{c}	75.00^{b}
Ethanol extract	25 .00^{a}	45.00^{a}	25.00^{a}	40.00^{a}	50.00^{b}	65.00^{a}
Carica papaya						
Water Extract	50.00^{c}	55.00^{d}	55.00^{c}	75.00^{d}	60.00^{c}	75.00^{b}
Ethanol extract	35.00^{b}	55.00^{d}	35.00^{b}	50.00^{b}	50.00^{b}	65.00^{a}
Control						
Water (alone)	100.00^{g}	100.00^{g}	100.00^{g}	100.00^{f}	100.00^{e}	100.00^{d}
Ethanol (alone)	96.00^{f}	85 .00^{d}	90.00^{e}	88.00^{f}	98.00^{d}	95.00^{e}
Mancozabe	25.00^{a}	40. 00^{a}	17.00^{a}	30.00^{a}	45.00^{a}	60.00^{a}

A - Pineapple sprayed with extracts before inoculating the pathogen. B - Pineapple inoculated with pathogen before spraying the plant extracts. Values are means of 3 replicates in two separate experiments. Values with same superscript are not significantly different ($P \leq 0.05$)

DISCUSSION

The pathogenicity test of fungal isolates from diseased pineapple fruits showed *R. stolonifer, A. niger* and *F. solani* as pathogenic organisms causing storage rot of pineapple with *A. niger* occurring most frequently and recording the highest disease incidence and severity than *R. solonfer* and *F. solani*. Pineapple fruits have been reported to be infected by a wide range of diseases such as *Phytophthora* rot and *Fusarium* rot (Evans *et. al.*, 2002). Black rot of pineapple caused by *Thielaviopsis pradoxa* and soft or wet rot caused by *Ceratocystis paradoxa* have been reported by Paulin-Mahady *et. al.* (2002). Akinmusire (2011) reported that *A. flavus, Rhizopus spp, Fusarium spp* and *Phytophthora spp* were the major cause of losses of *Ananas comosus* in storage. The differences recorded in the rot-causing organisms by different scientists may be due to different environmental and laboratory conditions under which the various researches were carried out.

The water and ethanol extracts of *M. oleifera, A. indica* and *C. papaya* exhibited varying levels of antimicrobial activity against the rot causing organisms of pineapple indicating that the pathogenic organisms showed varying levels of susceptibility to different active compounds of the plant materials with *R. stolonifer* being more susceptible followed by *A. niger* and *F. solani*. Both water and ethanol leaf extracts of the plant materials significantly reduced rot disease in pineapple caused the pathogenic organisms compared to the control experiment indicating antimicrobial potential of the plant materials. The ethanol extracts of the plant materials were more effective than water extracts in reducing the disease incidence and severity suggesting that solvent of extraction affected the antimicrobial activities of test plant materials with ethanol extracting more active ingredients from the test plants than water that led to the recorded higher fungitoxic activities of the ethanol extracts than water extracts of the plant materials (Amadioha, 2003; Amadioha and Markson, 2007; Amadioha, 2006; 2012). Ethanol leaf extracts of *A. indica* was more effective than other plant extracts and this was not significantly different to the synthetic fungicide (mancozade) in reducing the disease incidence in pineapple suggesting that the extract contained more active compounds than other plant materials which was comparable to the synthetic fungicide (mancozabe). Enyiukwu and Awurum (2013) reported that leaf extracts of *C. papaya* inhibited the development and spread of anthracnose in cowpea caused by *C. destructivum*, and the result was superior to synthetic fungicide. Application of ethanol and water leaf extracts of the plant materials before inoculation of pathogenic organisms recorded lower disease incidence than when applied after inoculating the pathogens indicating that the extracts are better used as protectant before infection and development of the disease than as eradicant when the disease has developed. Extracts of several plants have been used to control the rot development and spread in cassava (Amadioha and Markson, 2007; Amadioha and Opara, 2012; Amadioha, 2012)) and black rot of potato caused by *Rhizoctonia bataticola* (Amadioha, 2004). Leaf extracts of *C. papaya* inhibited the spore germination of *Colletotrichum destructivum* in culture and development and spread of anthracnose in cowpea caused by *C. destructivum*, and the results were superior to a synthetic fungicide (Enyiukwu and Awurum, 2013). The low disease incidence and severity recorded with the extracts both before and after inoculation of pineapple compared to the control experiment indicates that the test plants contain antimicrobial compounds that could be exploited as pesticides of plant origin in the control of postharvest microbial deterioration of pineapple fruits. The plant materials are readily available, biodegradable, cost effective and environmentally friendlier than synthetic pesticides which are not only hazardous to the environment and human but also scarce and expensive for resource poor farmers who produce over 95% of food consumed in Nigeria.

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