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# BIOCONTROL OF POST-HARVEST FUNGAL DISEASES OF CITRUS SCINENSIS (SWEET ORANGE) USING LEAF EXTRACTS OF AZADIRACHTA INDICA (NEEM) AND CHROMOLAENA ODORATA

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**ABSTARCT**

Post-harvest deterioration is a major problem of sweet orange (C. sinensis) production in Akwa Ibom State, Nigeria. Miicrobial infection of the fruits is mainly responsible. The present study was therefore, carried out to identify and biologically control the micro-organisms responsible for orange fruit rot during storage. Aqueous leaf extracts of Azadirachta indica and Chromolaena odorata were used as biological agents against fungal isolates. Samples of rotten orange fruits were collected from different markets across the state. Four fungal isolates (Penicillium digitatum, Aspergillus niger, Aspergillus flavus and Cladosporium herbarum ) obtained from naturally infected fruits were confirmed to be causal agents through pathogenicity testing. Phytochemical analysis of the extracts revealed higher amounts of polyphenols, flavonoids, saponin, tannin and alkaloids in A. indica compared to C. odorata. In-vitro investigations showed that 30% concentration of A. indica leaf extracts caused highest mycelial growth inhibition of the four pathogens (70, 75, 83 and 88% respectively) compared to the control, while extracts of C. odorata caused relatively lower inhibition of mycelial growth (50, 61, 61, 62% respectively) at the same concentration. Percentage inhibition increased with increase in extract concentration. These results indicate that aqueous leaf extract of A. indica is a better biocontrol agent of post-harvest orange fruit fungal diseases. Further studies are ongoing to test the validity of these results in the field.

**KEYWORDS**: Sweet orange, spoilage, fungi, leaf extract

## INTRODUCTION

Citrus sinensis is a medium size tropical plant generally cultivated in the Tropical, Sub-Tropical and Mediterranean regions. It is the most popularly cultivated fruits accounting to about 78.2% million metric tonnes in 1991; followed by grape, banana, and apple which were 55.9, 47.8 and 39.6 million metric tonnes respectively [1]. In Nigeria, commercial cultivation in large plantations is mostly within the middle belt. However, some Southern states and those in the far North also engage in *Citrus* large scale cultivation [2]. In the South-South region including Akwa Ibom cultivation is mainly by individual farmers.

Mature Citrus sinensis fruits are reported to contain 87.67% moisture, 11% carbohydrates with a calorific value of 48cal/100g [1]. These fruits are also rich in organic acids, essential oils beta carotenes, vitamin C, effective antioxidants and their pulps contain significant amounts of protein and soluble fibres [3]. The presence of flavonoids, carotenoids and limonoids in these fruits make them useful as anti-cancer, anti-viral as well as anti-inflammatory products [4]. On the other hand, *Citrus* fruits have high susceptibility to attacks by pathogenic fungi, due to their

low pH high moisture content and nutritional composition. These attacks result in fruit rots and other diseases which make them unsuitable for human consumption due to mycotoxin production .

Reports on Citrus post-harvest fungi rots are extensive and world-wide. A number of fungal species including Phythium, Phytophthora, Botryodiplodia, Aspergillus, Penicillium, Rhizopus, Glasdosporium, Mucor, have been associated with Citrus fruit rot [5,6,7]. Harvesting injuries, improper handling during transportation and poor storage facilities are general predisposing conditions for fruit rot development especially in economically stressed communities. In Nigerian markets, 20-90% fruits displayed for sale are reported to show symptoms of microbial infection [5]

Due to environmental concerns, the control of plant diseases is now focusing on the use of natural products in place of traditional chemicals with hazardous effects. This study was therefore, conducted to identify the pathogenic fungi and also to determine the possibility of using water leaf extracts from A. indica and C odorata as biocontrol agents of these pathogens.

## MATERIALS AND METHODS

### Sampling Sites

Five main markets were selected one each from the following locations: Ikono, Eket, Okobo, Ika and Uyo. Local Government Areas of Akwa Ibom State. These locations represent the North, South , East, West and Central regions of the State.

### Sample Collection

A total of 100 samples in batches of 20 were randomly selected from each market location. Infected orange fruits were identified by physical examination following the method described by [8] method. Fruits were said to be infected when they possess purple, dark brown or black rots symptoms. Healthy oranges were also collected to serve as controls. Percentage fruit infection was assessed to determine the severity or incidence of rot in each of the five markets. Sampling was carried out between March 2013 and March 2014.

### Isolation And Identification Of Fungal Isolates

Samples of apparently diseased fruits were surface sterilized by wiping with 85% ethanol and rinsed in three changes of sterile distilled water. These were then blotted dry and portions from the advancing edges of lesions were obtained using sterile knives. One gram of each cut portion was then homoginized using a sterile glass rod and a test tube in 9ml of sterile distilled water to produce a 101 concentration.

Potato Dextrose Agar plates containing Chloromphenicol (30mg/l) to prevent bacterial growth were inoculated with 0.1ml aliquots of homogenates and incubated at 25$^{o}$C for 7 days. Lactophenol cotton blue stain was employed and observations of morphological characteristics of isolates were made using the Olympus model light microscope. The identification of fungal isolates was carried out based on cultural and morphological features [9]

### Pathogenicity Test

Healthy oranges were surface sterilized and holes made using a 3mm sterile cork borer. 3mm portions obtained from advancing margins of PDA plates of each isolate were separately inserted into the holes and these holes were sealed with Vaseline jelly to prevent other microbes from entering the tissues. Both inoculated and uninoculated oranges were separately incubated in plastic containers at 25$^{o}$C for 7-14 days. Within this period, observations were made for growth of fungal colonies which were matched with the colonies used as sources of inoculum.

### Preparation And Analysis Of Plant Extract

Fresh leaves of A. indica and C. odorata were collected from around the Akwa Ibom State University Main campus and plant extracts prepared as described by [10] A portion of the extract was used for phyochemical screening following the method of [11]. Dilutions of 10, 20 and 30% concentrations (v/v) were prepared and used for bioassays.

### Effects Of Extracts On Pathogenic Fungi

The effects of leaf extracts on fungal pathogens was assessed based on mycelial growth as described by [10] The incubation was carried out at 25+2$^{0}$C and terminated at 7 days when the control mycelia had reached the edge of the Petri dish. The set up was carried out in triplicates. Percentage inhibition of mycelia growth was then determined using the formula:

$$\frac{P1 - P2}{P1} \times \frac{100}{1}$$

where P1= radial distance in negative control; P2 radial distance of pathogen with treatments.

### Statistical Analysis

All experiments were performed trice. Data were analyzed with Analysis of Variance

using MSTAT-C program version 2.10 [12]. The least significant difference (LSD) was used to test for significant differences between treatments at P≤0.05 [13]

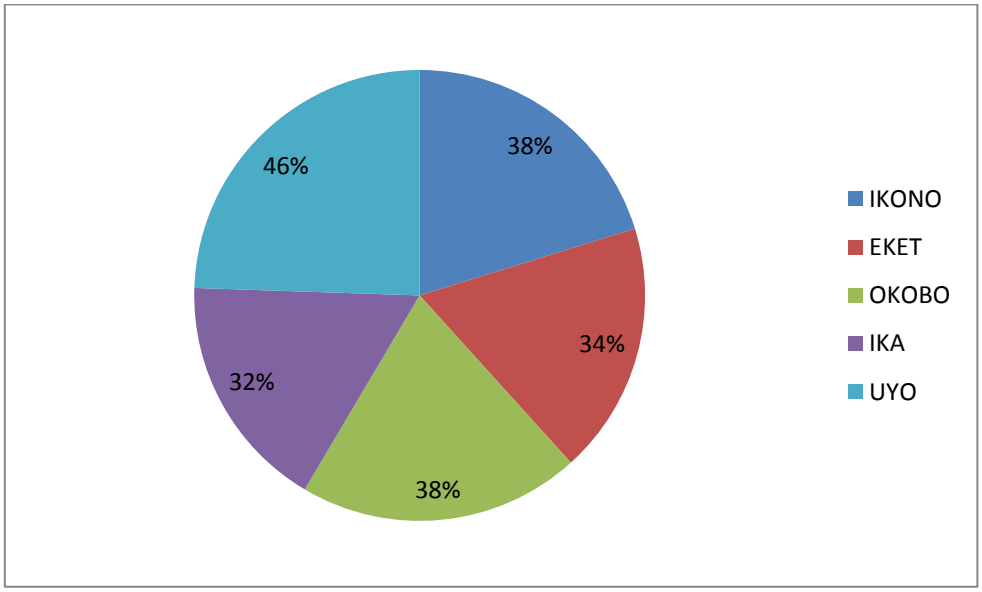
## RESULTS AND DISCUSSIONS

### Disease Incidence

The rate of Citrus sinensis postharvest fungal infection in five markets are shown in Figure 1

Ika market recorded the lowest percentage infection of 32 followed by Eket with 34%. Ikono, Okobo and Uyo had 38, 38 and 46% respectively. High incidences of *Citrus sinensis* post-harvest fruit rot ranging from 20-90% have been reported in other states of the country [5,14 and 15]. The high infection rates could be associated with poor postharvest handling, improper transportation processes and inadequate storage facilities. Ripe fruits are often packed in jute bags which could be stored for days before taken to the markets **Figure 2** Also, oranges are transported to the markets parked in open buses

**Figure 1:** Percentage infection of C. sinensis fungal rot in five market areas of Akwa Ibom State




In addition, fruits are often openly displayed for sale in the markets Figure 4 These conditions certainly favour fungal growth and consequent disease development. Uyo, a metropolitan city had the highest fruit rot incidence. Being the state capital, it has the largest market and most farmers/traders prefer transporting their produce to this centre from long distances. Sometimes these oranges are transported from other states and could take several days before getting to Uyo considering our deteriorated road conditions.

**Figure 2***: Jute bags packed with ripe Citrus fruits*

**Figure 3**: *A Bus Conveying Sweet Oranges To The Market*

**Figure 4:** *sweet oranges displayed for sale in the market*

**PATHOGENICITY TEST**

Four fungi isolated from naturally infected sweet orange fruits were confirmed causal agents of postharvest rot through pathogenicity test (Table 1).

**Table 1:** Fungal Isolates from five market areas in Akwa Ibom State

| Sampled Markets | Fungal Isolates | | | |
|---|---|---|---|---|
| | P. digitatum | A. niger | A. flavus | C. herbarum |
| Ikono | + | + | + | - |
| Eket | + | + | + | + |
| Okobo | + | + | + | - |
| Ika | + | + | - | + |
| Uyo | + | + | + | + |

(+ = present; - = absent)

P. digitatum and A. niger were found to be most prevalent as they were present in all the five sample sites. A. flavus was recorded in Ikono, Eket , Okobo and Uyo markets while C. herbarum was present in Eket Ika and and Uyo. All the four fungal pathogens were found in Uyo and Eket markets Table 1 The pathogens in this study have earlier been associated with sweet orange fruit rot in other parts of the country [5, 7, 14 and 16]. In other parts of the world, a number of other fungi species (Phytophthora, Rhizopus, Trichoderma, Curvularia, Botryodiplodia, Mucor, Gloesporium, Fusarium, Saccharomyces etc) have also been implicated in sweet orange postharvest rot [17, 18 and 19]. It is obvious that ripe sweet orange fruits are highly susceptible to a wide range of fungi. This may be attributed to their succulent tissues and high nutritional composition which forms growth medium for various pathogens.

**PLANT EXTRACT ANALYSIS**

Results of phytochemical analysis of A. indica and C. odorata leaf extracts are shown in Table 2. Leaf extracts of *A. indica* were found to contain relatively more of all the substances identified compared to extracts of C. odorata. Similar compounds were reported in extracts of *Theobroma cacao* [10].

**Table 2:** *Phytochemical constituents of extracts*

| Chemical compounds | Presence/Absence | |
|---|---|---|
| | A. Indica | C. Odorata |
| Polyphenols | +++ | + |
| Flavonoids | +++ | ++ |
| Sapronin | +++ | ++ |
| Tannin | ++ | + |
| Alkaloids | ++ | + |

+ = low; ++ = moderate ; +++ = high content

**LEAF EXTRACTS BIOASSAYS**

The bioassay results indicated antifungal activities in the two extracts with A. indica being more effective in inhibiting mycelia growth when compared to C. odorata ( Figs. 4 and 5). Percentage inhibition was also found to increase with increase in concentration of extracts. At 30% concentration, A. indica recorded 70, 75, 83 and 88% inhibition respectively for the four pathogens while C. odorata at the same concentration had 50, 61, 61 and 62% inhibition respectively. The higher inhibitory effects of A. indica extracts could be associated with higher contents of the five phytochemical compounds as compared to relatively lower contents in C. odorata.

A number of crude plant extracts including *A.* indica, Ocimum graticimum, Allium cepa, Allium sativum, Theobroma cacao, Aframomum melequeta, C. odorata,Mangifera indica, Vernonia amygdalina etc have also been reported as alternative plant disease control agents [5, 6, 7, 10, 20 and 21]. These extracts are reported to contain phytochemical substances identical to those recorded in this study. They are considered environmentally friendly and therefore, preferred to synthetic pesticides that have been traditionally used in the control of plant diseases. Apart from being hazardous, synthetic pesticides are expensive and most farmers are unable to afford these products. The fact that plants used in these study are easily available, couple with their simple methods of extraction and application, makes them potential cheap alternatives for the control of fungal sweet orange postharvest rot. However, further investigations are required to confirm their field applications.

**Figure 5:** Inhibitory effect of A. Indica leaf extract on mycelia growth of fungal pathogens

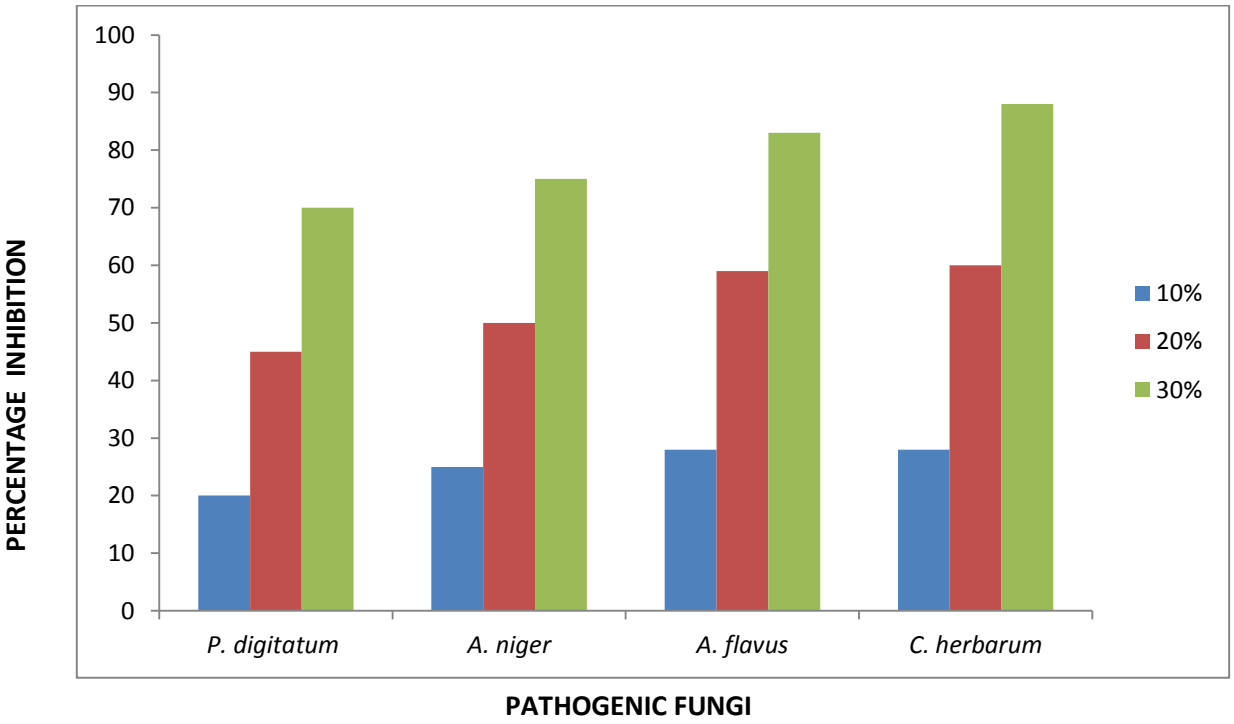
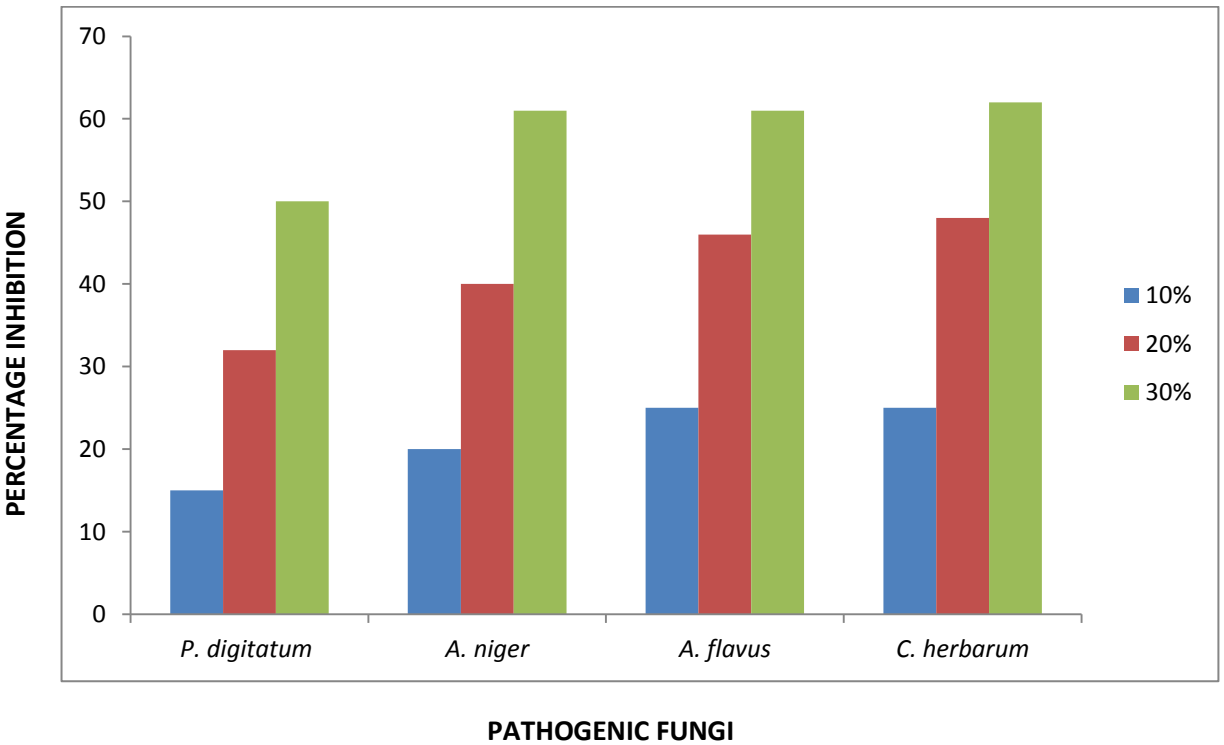



**Figure 6:***Inhibitory effect of C. Odorata leaf extract on mycelia growth of fungal pathogens*




## CONCLUSION

Leaf extracts of *A. indica* and *C. odorata* have great biopesticide potentials for the control of post-harvest fungal fruit diseases of sweet orange.

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