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Evaluation of some aqueous plant extracts used in the control of pawpaw fruit (*Carica papaya* L.) rot fungi

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ABSTRACT

Objectives: To isolate, identify and establish the pathogenicity of fungi associated with post-harvest rot diseases of pawpaw fruits, and to determine the effect of various concentrations of crude extracts from the leaves of *Carica papaya, Chomolaena odorata*, and *Acalypha ciliata* on the growth of the pathogenic fungi.
Methodology and results: All experiments were carried out under laboratory conditions. Fungi associated with diseased pawpaw fruits which were naturally infected and obtained from the nearby market were isolated using the blotter method and grown on Potato dextrose broth (PDB). Plant materials including *Chromolaena odorata*, *Acalypha ciliate* and *Carica papaya* were obtained and their ingredients extracted by slurry and filtration. Anti-fungal activities of the aqueous extracts of the leaves were determined by measuring the mycelia dry weight. The fungi isolated (and their frequency of occurrence) included *Aspergillus niger* (26.83%), *Botryodiplodia theobromae* (31.71%), *Fusarium solani* (39.02%) and *Penicillium* sp (2.44%). The results showed that all the tested concentrations (10, 20 and 30%), significantly ($p<0.05$) reduced the mycelial growth of the fungi in-vitro. The fungicidal effect of *A. ciliata,* was greater than that of *Chromolaena odorata* and *Carica papaya*.
Conclusions and application of findings: The information obtained in this research could be applied in the control of fungal diseases of fruits.
Key words: *Carica papaya,* storage fruit diseases, *Aspergillus niger, Botryodiplodia theobromae*, *Fusarium solani.*

INTRODUCTION

Carica papaya is the most important species within the family Caricaceae being cultivated widely for consumption as a fresh fruit and used for drinks, jams, candies, or as dried and crystallised fruit. The unripe fruits are sometimes eaten as vegetables when cooked (Vickery and Vickery, 1979). The fruits contain papain, a proteolytic enzyme used industrially in beverages, food, pharmaceutical industries, including in the production of chewing gums, tenderising meat and drug preparation for various digestive ailments and for the treatment of gangrenous wounds. It is also an important component of some toothpaste used for curing pyrorrhoea, a disease of gums (Kochhar 1986). The fruits are high in moisture, ash, crude fibre, carbohydrate and calorie as well as rich in vitamins A, C and B-carotene. The fruits also contain toxicants such as oxalates, hydrocyanic acid and phytic acid (Umoh, 1998).

A number of fungal diseases in pawpaw fruits have been reported including Fusarium rot caused by *Fusarium solani* (Mart) Sacc. (Dathak *et al.,* 1976) and Rhizopus rot caused by *Rhizopus stolonifer* (Ehrenb. Ex. Fr.) Lind (Snowdon, 1990). T *Fusarium solani* can cause a destructive rot in pawpaw in all producing areas with great losses

reported from Hawaii, the Philippines, India, Pakistan, Israel and Nigeria. Aspergillus rot of pawpaw may be caused by various species of *Aspergillus*, e.g. *Aspergillus flavus* and *A. niger.* These fungi are commonly associated with pawpaw fruits causing post harvest diseases. Pawpaw fruits are contaminated at harvest and decay is favoured by warm, humid condition which encourages the development of abundant spores (conidia) on the cut surface (Snowdon, 1990).

The hazards involved in using chemical pesticides and the development of resistance to synthetic fungicides by plant pathogenic organisms, make alternative controls desirable. Furthermore, synthetic fungicides are expensive and inaccessible to indigenous farmers who are the bulk producers of pawpaw Nigeria (Amadioha, 1998; Onuegbu et al., 2001). A natural plant product with fungicidal properties could be less expensive and more environmental friendly than synthetic fungicides.

Medicinal plant materials have been successfully used for the treatment of fungal and bacterial infections in humans (Akinyosoye and Oladummoye, 2000), suggesting that some plant materials may also possess antifungal and antibacterial constituents that are useful in controlling plant diseases (Amadioha, 1998). Previous reports (Akpomedaye and Ejechi, 1998; Ejechi and Ilondu, 1999; Ejechi et al., 1999) show that spices, herbs and other plant materials possess antifungal activity. Akinyosoye and Oladunmoye (2000) have reported the antifungal efficacy of stem and leaf-extracts of *Mirabilis jalapa* in reducing mycelial growth of four different strains of fungi. The legendary medicinal qualities of the neem tree have been known for a long time and their aqueous leaf extracts have systemic action (Egunjob and Onoyemi, 1981; Sownumi and Akinusi, 1983). Therefore, the expression of fungitoxic activities by plant extracts is an indication that such plants could be used as fungicides by peasant farmers who cannot afford the costly synthetic agrochemicals to control fungal diseases on attacking their crops.

Fruits have not been fully included in the diets of Nigerian people in spite of their desirable attributes. This is due to ignorance of the nutritive values of fruits, rising cost of fruits, and problems of storage of these perishable commodities due to diseases (Umoh, 1998). Several attempts have been made to use fungicides such as benomyl to control the main diseases. However, chemical control of disease has several drawbacks, and this has stimulated the search for more acceptable alternative natural products, which are less phytotoxic and biodegradable.

This work was undertaken to isolate, identify and establish the pathogenecity of fungi causing post-harvest rot of pawpaw fruits. In addition, the effect of various concentrations of crude extracts from the leaves of *Carica papaya, Chomolaena odorata*, and *Acalypha ciliata* on the growth of the isolated fungi was determined.

MATERIALS AND METHODS

Collection of pawpaw fruits: Both healthy and diseased pawpaw fruits were purchased from Abraka market in Delta State, Nigeria

Isolation and identification of fungi from diseased fruits: The fungi associated with diseased pawpaw fruits were isolated using the blotter method as recommended by the International Seed Health Testing Association (1976). The pawpaw fruits were cut into sections with a sterilized knife. The sectioned fruits were surface sterilized with 70% ethanol for three minutes and rinsed with three charges of sterile distilled water to remove surface contaminants. The sterilized sectioned fruits were dried between filter papers and plated on three layers of filter papers moistened with sterile distilled water in already sterile Petri-dishes (disposable) and incubated at room temperature (28 ± 2°C) for 7 days. After incubation, the plates were observed under binocular microscope for fungal growth. Identification of the isolated fungi was carried out based on their cultural characteristics and with the help of identification scheme of Bernet and Hunter (1972). To obtain pure cultures of the isolated fungi, a sterile inoculating needle was used to transfer the fungal spores from filter papers in the Petri-dish and inoculated on acidified potato dextrose agar medium (APDA). Each fungus was plated at the center of the

Petri dishes. The name of each fungus and dates of inoculation were labelled on the plates. They were incubated at room temperature (23 ± 2°C) in a laminar flow for seven (7) days and their growth observed.

Determination of the frequency of occurrence of the isolated fungi: The frequency of isolations of the different types of fungi associated with pawpaw fruit rot diseases was determined. The number of times each fungus was encountered was recorded. The percentage frequency of occurrence was calculated formulas follows:

$$\frac{\text{Number of times a fungus was encountered}}{\text{Total fungal isolations}} \times 100$$

Preparation of media (PDA and PDB): To prevent bacterial growth, the PDA medium was modified by adding 3 drops of lactic acid and shaken before pouring into petri-dishes for solidification.

To prevent bacterial growth, 0.2g of streptomycin was added to the potato dextrose broth.

Pathogenecity test: To ascertain pathogenecity of the various fungi isolated, freshly harvested matured unripe fruits were surfaced sterilized with 70% alcohol and rinsed in sterile distilled water. With a 3mm diameter sterile cork borer, 2cm long cylindrical cores were removed from each fruit; discs of 7-day old cultures of each isolate were removed from agar plates and placed in the bored holes on each fruit. Vaseline jelly was smeared to completely seal each hole. The control was inoculated with disc of solidified potato dextrose agar medium. In another set, spore suspensions were prepared by adding l0ml of sterile distilled water to the different isolates and the spores gently dislodged using a sterilized glass rod. The suspensions were then poured into sterile bottles and inoculation was carried out by surface-run off. This was done using a sterile syringe and simply spraying the spore suspensions onto the fruits surfaces. Sterile distilled water was used to spray the control fruits. After one week of incubation at room temperature, (28 ± 2°C) the inoculated fruits were incised and observed for disease development . Rot symptoms developed with different fungal isolates were compared to the natural original rot.

Collection and extraction of plant materials: The plant materials used were *Chromolaena odorata, Acalypha ciliata* and *Carica papaya* . Fresh healthy leaves of the plant materials were collected, washed, dried at 28°C and blended into powder. One hundred ml of distilled water was added to 10, 20 and 30g of the powdered leaf materials to form slurry and to obtain three different concentrations. Each suspensions of the slurry were vigorously shaken to effect dissolution and the suspensions were then filtered through a filter paper to obtain extracts (Owolade and Osikanlu, 1999).

Anti-fungal activity of the extracts: The effect of the extract was determined by measuring the mycelial dry weight. Fifty ml of potato dextrose broth (PDB) was poured into each flask containing different concentrations (0, 10, 20 and 30) of the respective extracts (2ml each). With a sterile cork borer (3mm) mycelia disc of 7 day old cultures of the isolates were inoculated in the flask and incubated at 28± 2°C. After 7 days the different fungi from the different broths, were taken on dried and weighted filtered papers in a desiccator. After this, fungal mycelia were dried at 70°C for 24 hours and the weight was recorded.

Inhibition of fungi by various concentrations was calculated as:

$$\frac{100 - \text{Weight of fungus in extract}}{\text{Weight of fungus in PDB}} \times 100$$

(Adejumo et al.2000)

Data analysis: Two way-Analysis of Variance (ANOVA) was applied in comparing the different isolates and concentrations.

RESULTS

The fungi isolated from rotted pawpaw fruits were *Aspergillus niger, Fusarium solani, Botryodipdia theobromae and Penicillium* sp. *F. solani* occurred more frequently with 39.02%, followed by *B. theobromae* with 31.71%, *A. niger* with 26.83% and the least was *Penicillium* sp. with 2.44% (table 1). The pathogenecity tests of the isolates on intact fruits surfaces showed no visible symptoms. On the other hand, pathogenecity of the wounded fruits being inoculated with mycelial discs of the isolates showed that *F. solani, B. theobromae* and *A. niger* were pathogenic while *Penicillium* sp showed no visible symptoms. All plant extracts tested significantly (p<0.05) reduced the mycelial growth of all the fungi in the broth medium at all concentrations (table 2). However the effectiveness of the plant extracts increased with increased concentration and this was also statistically significant (p<0.05).

Table 1: Frequency of occurrence of fungi from rotted pawpaw fruit.

Fungi isolated	Number of times isolated	Percentage frequency (%)
A. niger	44	26.83
B. theobromae	52	31.71
F. solani	64	39.02
Penicillium	4	2.44

At all concentrations, the extracts inhibited growth of *B. theobrornae* more than that of *A. niger* and *F. solani.* However, the toxicity of *A. ciliata* was greater than that of *C. papaya* and *C. odorata* in the percentage growth inhibition of the isolates (Table 3). For all the concentrations of plant extracts used in this work, there was almost a constant 100% inhibition approximately.

Table 2: Mycelial dry weight (gm) of fungi isolated from pawpaw fruits when exposed to varying concentrations of various plants leaves extracts

Conc. (%)	***C.papaya***			***C. odorata***			***A. ciliata***		
	A. niger	*F. solani*	*B. theobromae*	*A. niger*	*F. solani*	*B. theobromae*	*A. niger*	*F. solani*	*B. theobromae*
0	2.00	1.30	2.01	2.00	1.30	2.01	2.00	1.30	2.01
10	0.11	0.09	0.06	0.10	0.08	0.04	0.08	0.05	0.04
20	0.07	0.05	0.03	0.06	0.04	0.03	0.05	0.03	0.03
30	0.04	0.04	0.02	0.04	0.02	0.02	0.03	0.02	0.01

Table 3: Percentage growth inhibition of fungal isolates from pawpaw fruits after exposure to varying concentrations of *leaf* extract of various plants (after seven (7) days)

Conc. (%)	***C.papaya***			***C. odorata***			***A. ciliata***		
	A. niger	*F. solani*	*B. theobromae*	*A. niger*	*F. solani*	*B. theobromae*	*A. niger*	*F. solani*	*B. theobromae*
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	95.5	93.08	97.01	95	93.85	98.01	96	96.15	98.01
20	96.5	96.15	98.51	97	96.83	98.51	97.5	97.69	98.51
30	98.0	96.92	99.05	98.0	98.46	99.01	98.5	98.46	99.50

DISCUSSION

The present study showed that a number of fungi are associated with post-harvest rot diseases of pawpaw fruits. These fungi include *Aspergillus niger*, *Fusarium solani*, *Penicillium* sp. and *Botryodiplodia theobrornae*. They have been previously reported as fruit rot pathogens of pawpaw fruits (Pathak et al., 1976; Alverez and Nishijima, 1987).

It was observed that the presence of wounds on the fruits for disease to occur as intact fruits showed no infection when inoculated with spore suspensions of the isolates. This observation is similar to that of Nishijima et al. (1990) showing that *Rhizopus* requires a break in the cuticle for successful infection to occur. Unripe fruits inoculated with mycelial discs of the isolates did not show disease symptoms until they were ripe. This is inline with the findings of Elmoussaoui et al. (2001) who reported that the green fruits of pawpaw contain papain, an enzyme which could possibly inhibit *Fusarium sp. A. niger, F. solani* and *B. theobromae* were most pathogenic while *Penicillium* was not pathogenic on pawpaw. Possibly, *Penicillium* acted as a secondary invader on lesions caused by other fungi; hence it could not reproduce the post-emergence rot on pawpaw fruits irrespective of the inoculum method employed. This observation agrees with the reports of Flentje (1965) and Wilhelm (1967) showing that such organisms, when tested against the host in the absence of the primary pathogen, would have no effect. Also Johnson et al. (1978) and Colyer (1988) reported only

mild virulence and considered the fungus to be a secondary invader.

Investigation into the antifungal properties of *C. odorata, C. papaya* and *A. ciliata* on the growth of the isolates shows that these plants' crude extracts possess some inhibitory components which cause significant reduction in mycelial growth of the fungi. This agrees with the results of Amadioha (1998), Owolade and Osikaniu (i999) and Adejumo et al. (2000) who reported the efficacy of extracts from *C. papaya*, *A. ciliate*, *C. odorata*, among other extracts in reducing the mycelial growth of *Erysiphe cichoracearum, Collectotrichum capsici* and *Protomycopsis phaseoli*, which compared favourably with the chemical pesticides Benlate and Ridomil. Akpa et al. (1991) reported a significant inhibitory property of neem (*A. indica*) extracts on mycelial growth of *Collectotrichum graminicola* just as Amuchi (1989) found the extracts of *Ocinum gratissinum* to reduce the radial growth of *Rhizopus* spp. Comparing among the plant extracts, *A. ciliata* was most effective in inhibiting the fungi in-vitro, followed by *C. odorata* and *C. Papaya*, considering their various inhibitions at the different concentrations. Investigation is therefore suggested purify and characterize the active components of these plants extracts with the view to incorporating it into ointment for in-vivo application Efforts should also be made to screen the flowers, stem and roots of these plants for possible antimicrobial activity.

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