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Bio-Treatment Of Fruitlet Core Rot Associated With Fungi-Induced Postharvest Deterioration In Ananas Comosus Linn. (Pineapple)

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***Abstract:** Pineapple is the third most important commercial fruit in the global market and its availability is directly affected by disease. Control of microorganisms especially pathogens is being plagued by resistance hence the need for constant search for new derivatives especially those from natural sources. Therefore, the current study seeks to investigate the effects of Syzygium aromaticum (S. aromaticum) and Acalypha wilkensiana (A. wilkensiana) on fungal pathogens associated with fruitlet core rot of pineapple. Pineapple samples with symptoms of fruitlet core rot were inoculated into freshly prepared Potato Dextrose Agar for isolation of pathogenic fungi. Pathogenicity test was carried out by inoculating the isolated fungi into healthy pineapple fruits. Methanolic and aqueous (cold and hot) extracts of A. wilkensiana (leaves) and S. aromaticum (buds) were obtained and tested against the isolated fungi. Aspergillus niger, Fusarium sp. and Pencillium sp. which isolated from rotten pineapple and were proven to be causal agents of spoilage, with Fusarium sp. being the most virulent. (100, 250, 500 and 750)mg/mL of aqueous and methanolic extracts of both plants significantly reduced radial mycelial growth of the isolates in in vivo tests. The extracts of the 1 plants were fungitoxic to the rot pathogens and can be used to control the growth of the spoilage fungi.*

***Keywords:** Pathogenic, in-vitro, in-vivo, fungicide, fungitoxic*

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I. INTRODUCTION

Pineapple (*Ananas comosus* L.) classified under the family Bromeliaceae is an economically important crop that plays a key role in rural development and improves general well-being due to the nutrients present (All Africa, 2011). Hence it plays a role in food security and job creation. Baruwa (2013) stated that pineapple is the most important commercial fruit after banana and citrus in the world, dominating 50.7% of the global fruit market. It is cultivated in tropical and subtropical countries in America, Asia and Africa for local as well as for foreign exchange earnings (Bartholomew *et al*., 2003; Elss *et al*., 2005; FAO, 2004 and 2005).

Postharvest disease of pineapple poses a great challenge globally occasioned by its high water content, susceptibility to injury and microbial infection. In other to reduce postharvest loss of pineapple, chemical control methods have been employed but they are restricted due to their possible carcinogenicity, long degradation, environmental pollution and their harmful effects on human beings. The use of biological control such as plant based extracts are being encouraged since they are economically feasible, host specific and has little or no side effect for prolonging its shelf-life and ensuring the availability of fresh healthy pineapple. Therefore, the current study seeks to investigate the effects of *Syzygium aromaticum* and *Acalypha wilkensiana* as control methods of fungal pathogens associated with fruitlet core rot of pineapple.

II. MATERIALS AND METHODS

A. SAMPLE COLLECTION

Fresh and healthy leaves of *Acalypha wilkensiana* were obtained from the Botanical Garden, University of Ibadan, Nigeria and fruits of *Syzygium aromaticum* were purchased from Bodija market in Ibadan, Oyo State, Nigeria. The plant materials were authenticated in the Herbarium unit of Botany Department, University of Ibadan. Pineapple fruits with symptoms of fruitlet core rot were purchased from Oje market and Bodija markets in Ibadan, Oyo state, Nigeria.

B. ISOLATION AND IDENTIFICATION OF FUNGI

Diseased fruits were washed under running tap and surface sterilized with 70% ethanol for 30 seconds to remove surface contaminants. It was then rinsed in three changes of sterile distilled water and then blotted dry with sterile filter paper. The infected parts were cut into small fragments and plated directly onto freshly prepared Potato Dextrose Agar (PDA). The plates were incubated at 28 ± 2^0C for 3-5days and were examined daily. The mixed cultures were subcultured until pure cultures of the isolates were obtained and maintained on PDA slants in McCartney bottles (Odebode *et al*., 2006). The fungi isolates were identified based on their colonial morphology and microscopic characteristics according to the identification procedure used by Odebode *et al*. (2006).

C. PATHOGENICITY TEST

The modified method of Chukwuka *et al*. (2010) was adopted. Healthy pineapple fruits were surface sterilized and a hole of 5mm diameter was aseptically bored into each fruit using a cork borer. Mycelial plug of 5-day-old culture was cut from the active growing part of the colony and inserted into the hole. The wound was sealed with petroleum jelly while control samples were treated in the same manner with inoculated PDA. The treated samples and control were placed individually in sterile polyethylene bags each moistened with wet balls of absorbent cotton wool to create a humid environment and incubated at 28 ± 2°C for 11days. At 2-day intervals, the samples were sectioned through the site of inoculation and examined for lesion development. Infected portions were aseptically transferred onto freshly prepared PDA.

D. EXTRACTION PROCEDURE

The modified method of Okigbo and Ogbonnaya (2006) was used. Methanolic and aqueous (cold and hot) extracts of ground fruits of *Syzygium aromaticum* were prepared by weighing 75g, 50g, 25g and 10g of the fruit into 100ml of hot sterile distilled water, cold sterile distilled water and methanol separately in 250ml beakers. The set-up was stirred vigorously, allowed to stand for 24 to 96 hours with intermittent shaking and filtered three times using sterile muslin cloth. Two hundred grams (200g) of the leaves of *Acalypha wilkensiana* were weighed and ground with 200ml of methanol (methanol extraction) and sterile distilled water (aqueous extraction) separately with a sterile electric blender and then filtered 3 times using sterile muslin cloth. The filtrates were stored in refrigerator at 4°C prior to use.

E. IN-VITRO BIOCONTROL OF THE FUNGAL PATHOGENS USING PLANT EXTRACTS

The method of Odebode *et al*. (2006) was adopted. Aseptically, 1 ml of each plant extract at different concentration (750mg/mL, 500mg/mL, 250mg/mL, and 100mg/mL) was pipetted into sterilized Petri dish, overlaid with 10 ml of molten potato dextrose agar, and mixed thoroughly. After the agar solidified, mycelial plug of 5-day-old culture was cut from the active growing portion of the mycelium and placed in an inverted position on the already prepared media. The culture plates were incubated at 28 ± 2^0C in an incubator. The diameter of growth of the pathogen was measured daily for 7 days. All the treatments were carried out in triplicates. Sterile distilled water and methanol were both used as control in the determination of radial growth of the fungal pathogens.

F. IN-VIVO BIOCONTROL OF THE FUNGAL PATHOGENS USING PLANT EXTRACTS

A modified method of Odebode and Che (2001) was adopted. Healthy pineapple fruits of approximately equal sizes and physiological maturity were surface disinfected by swabbing with cotton wool soaked with 70% alcohol, rinsed with three changes of sterile distilled water and blotted dry with sterile filter paper.

A hole was aseptically bored into each healthy surface sterilized fruit with a 5mm diameter sterile cork borer. Ten millilitres (10mL) of extracts at different concentration (750mg/ml, 500mg/ml, 250mg/ml or 100mg/ml) was pipetted into each hole. Thereafter, mycelial plug of 5 day old culture was cut from the edge of the colony and inserted aseptically into the holes and wound was sealed with petroleum jelly. Incubation was done at 28±2^0C for a period of 7 days. Three (3) sets of control were made: control A (fungal pathogen + distilled water), control B (fungal pathogen + methanol) and control C (fungal pathogen alone) respectively. All the treatments and controls were carried out in triplicates. At the end of incubation period, cuts were made along the plane of inoculation on each fruit using a sterile knife and rot was assessed by measuring the diameter of rot in mm; records were kept for each fungal isolates at each concentration of the extract used.

III. RESULTS

Fungal pathogens isolated from fruitlet core rot of pineapple were identified as:

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Aspergillus Niger

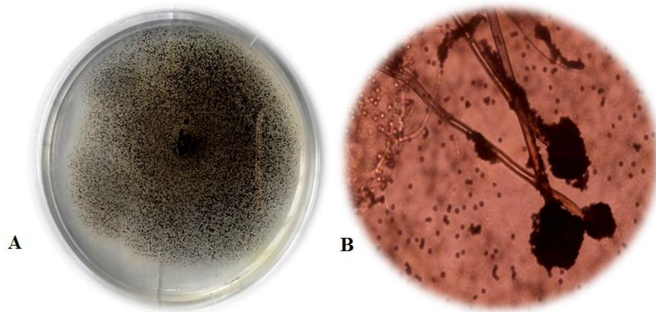

Plate 1: (A) Colonial morphology on PDA and (B) Photomicrograph of Aspergillus niger

Penicillium sp.

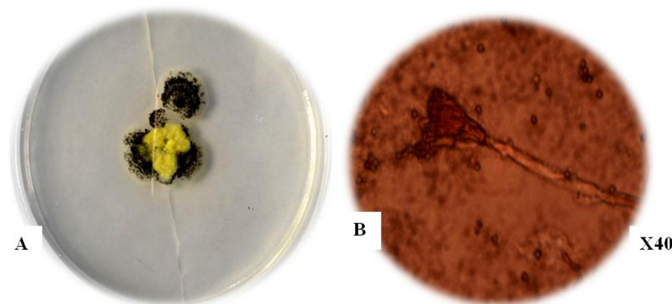

Plate 2: (A) Colonial morphology on PDA and (B) Photomicrograph of Penicillium sp.

Fusarium sp.

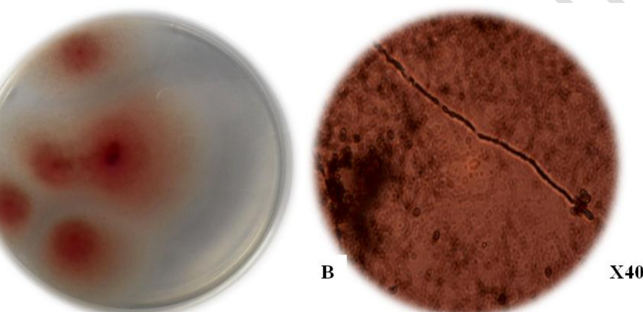

Plate 3: (A) Colonial morphology on PDA and (B) Photomicrograph of Fusarium sp.

All the fungal isolates re-produce the disease symptoms associated with fruitlet core rot of pineapple when re-inoculated into healthy (disease-free) pineapple samples as shown in (Table 1) with *Fusarium* sp. being the most virulent of the three isolates and *Aspergillus* sp being the least virulent. The results also showed (Fig 1) that fungal deterioration of the fruit began as early as on Day 3 with a climax of 40%, 78% and 100% deterioration by *Aspergillus, Penicillium* and *Fusarium* respectively on the 13th day.

	Level of Virulence						
Pathogen	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**	**Day 13**
A. niger	-	+	+	+	+	+	+
Penicillium sp	-	+	+	++	++	++	++
Fusarium sp	-	+	++	++	+++	+++	+++

Key:
+ = Mildly Virulent
++ = Moderately Virulent
+++ = Severely Virulent
-= Avirulent

Table 1: The pathogenicity test conducted for fungal species isolated from diseased pineapple fruits with fruitlet core rot symptoms

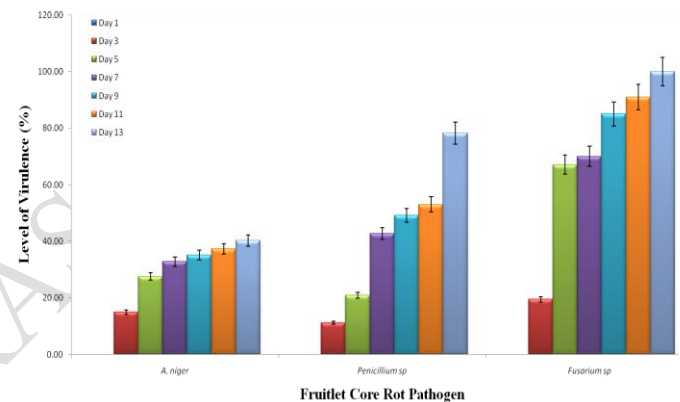

Figure 1: The percentage level of virulence of the re-inoculated pathogens in healthy pineapple fruits

The radial mycelia growth of the isolated fungal pathogens at 100mg/mL concentration of the plant extracts (Table 2). The aqueous extracts seemed to have greater efficiency at this concentration. At day 3, it was observed that cold water extract of *Syzygium aromaticum* had significant reduction on the radial mycelia growth of *Fusarium* sp. (0.10 ± 0.17) as compared to the control (2.50 ± 0.00) At the same day, it was also observed that cold and hot water extracts of *Acalypha wilkensiana* had significant reduction in the radial mycelia growth of *Fusarium* sp. (0.70 ± 0.13 and 1.18 ± 0.00) respectively as compared to the control *i.e Fusarium* sp. (2.50 ± 0.00) .

At day 7, it was observed that cold water extract of *S. aromaticum* had significant reduction on the same pathogen (*Fusarium* sp.) (0.43 ± 0.38) while hot water extract of the same plant was active in the reduction of radial mycelia growth of *A. niger*, *Fusarium* sp., and *Pencillium* sp. (1.28 ±0.36, 2.07 ± 0.45 and 0.37 ± 0.40) respectively as compared to the individual control. However, cold water extract of *A. wilkensiana* had significant reduction in the radial mycelia growth of *Fusarium* sp. (1.82 ± 0.53) and *Pencillium* sp. (0.70 ± 0.24) respectively as compared to the individual control while hot water extract of the same plant was active in the reduction of radial mycelia growth of *A. niger* (1.80 ± 0.00) as compared to the control.

Plant Extract	Solvent	Pathogens	Radial mycelia growth (cm) Day 3	Day 5	Day 7
Syzygium aromaticum	Cold Water	*Aspergillus niger*	1.32±1.71^{b}	1.90±1.77^{cde}	3.40±0.58^{ab}
	Cold Water	*Fusarium* sp	0.10±0.17^{d}	0.17±0.29^{fg}	0.43±0.38^{ijk}
	Cold Water	*Penicillium* sp	0.58±0.53^{cd}	2.22±1.62^{cd}	2.68±1.44^{bcde}
	Methanol	*Aspergillus niger*	0.19±0.17^{d}	0.50±0.44^{fg}	0.97±0.58^{hij}
	Methanol	*Fusarium* sp	0.45±0.00^{cd}	2.00±0.00^{cde}	3.00±0.00^{abcd}
	Methanol	*Penicillium* sp	2.50±0.00^{a}	2.70±0.00^{abc}	3.00±0.00^{abcd}
	Hot Water	*Aspergillus niger*	0.67±0.13^{bcd}	1.03±0.41^{fg}	1.28±0.36^{gh}
	Hot Water	*Fusarium* sp	0.98±0.31^{bc}	1.92±0.45^{cde}	2.07±0.45^{ef}
	Hot Water	*Penicillium* sp	0.00±0.00^{d}	0.00±0.00^{g}	0.37±0.40^{jk}
Acalypha winkensiana	Cold Water	*Aspergillus niger*	1.82±1.60^{b}	2.72±1.73^{abc}	2.93±1.59^{abcd}
	Cold Water	*Fusarium* sp	0.70±0.13^{bcd}	1.40±0.23^{ef}	1.82±0.53^{fg}
	Cold Water	*Penicillium* sp	0.23±0.21^{d}	0.50±0.17^{fg}	0.72±0.24^{ij}
	Methanol	*Aspergillus niger*	1.35±0.00^{b}	2.05±0.00^{cd}	2.35±0.00^{def}
	Methanol	*Fusarium* sp	0.45±0.00^{cd}	2.00±0.00^{cde}	3.00±0.00^{abcd}
	Methanol	*Penicillium* sp	2.75±0.00^{a}	3.18±0.00^{ab}	3.48±0.00^{a}
	Hot Water	*Aspergillus niger*	0.75±0.00^{bcd}	1.60±0.00^{ef}	1.80±0.00^{fg}
	Hot Water	*Fusarium* sp	1.18±0.00^{b}	2.28±0.00^{bcd}	2.58±0.00^{cde}
	Hot Water	*Penicillium* sp	3.13±0.55^{a}	3.43±0.38^{a}	3.28±0.13^{abc}
Control	Methanol	*Aspergillus niger*	0.00±0.00^{d}	0.30±0.00^{fg}	1.10±0.00^{hi}
	Water	*Fusarium* sp	2.50±0.00^{a}	2.70±0.00^{abc}	3.00±0.00^{abcd}
	Methanol	*Penicillium* sp	0.10±0.00^{d}	0.00±0.00^{g}	0.95±0.00^{hij}
	Water	*Aspergillus niger*	1.35±0.00^{b}	2.05±0.00^{cd}	2.35±0.00^{def}
	Methanol	*Fusarium* sp	0.00±0.00^{d}	0.00±0.00^{g}	0.30±0.00^{jk}
	Water	*Penicillium* sp	0.45±0.00^{cd}	2.00±0.00^{cde}	3.00±0.00^{abcd}

Table 2: The radial mycelia growth of the isolated fungal pathogens at 100mg/mL concentration of the plant extract

Means with the same alphabets down the column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only

The radial mycelia growth of the isolated fungal pathogens at 250mg/mL concentration of the plant extracts (Table 3). At day 3, it was observed that cold water extract of *S. aromaticum* significantly reduced radial mycelia growth of *A. niger* and *Fusarium* sp. (0.37 ± 0.40 and 0.00 ± 0.00) respectively as compared to the individual control i.e (1.35 ± 0.00) and (2.50 ± 0.00) while hot water extract of the same plant significantly reduced *A. niger, Fusarium* sp. *and Pencillium* sp. (0.68 ± 0.16, 1.00 ± 0.41 and 0.00 ± 0.00) respectively as compared to the individual control.

At day 5, it was also observed that cold water extract of *S. aromaticum* had significant reduction in the radial mycelia growth of *A. niger, Fusarium* sp. and *Pencillium* sp. (0.50 ± 0.50, 0.00 ± 0.00 and 1.07 ± 0.43) respectively as compared to the individual control (2.05 ± 0.00, 2.70 ± 0.00 and 1.20 ± 0.00) respectively It was further observed at the same day that cold water extract of *A. wilkensiana* had significant reduction on the radial mycelia growth of *Fusarium* sp. (0,58 ± 0.52) and *Pencillium* sp. (0.38 ± 0.34)as compared to the control i.e *Fusarium* sp. (2.70 ± 0.00) and *Pencillium* sp. (2.00 ± 0.00) .

At day7, it was observed that cold water extract of *S. aromaticum* was active in the reduction of *A. niger* (1.17 ± 0.63) and *Fusarium* sp. (0.65 ± 0.61) as compared to the control *A. niger* (2.35 ± 0.00) and *Fusarium* sp. (3.00 ± 0.00). It was further observed that cold water extract of *A. wilkensiana* was active in the reduction of radial mycelia growth of *Fusarium* sp. and *Pencillium* sp. (1.05 ± 0.3 and 0.70 ± 0.17) respectively as compared to the individual control (3.00 ± 0.00 and 3.00 ± 0.00) respectively

Plant Extract	Solvent	Pathogens	Radial mycelia growth (cm) Day 3	Day 5	Day 7
Syzygium aromaticum	Cold Water	*Aspergillus niger*	0.37±0.40^{h}	0.50±0.50^{h}	1.17±0.63^{fg}
	Cold Water	*Fusarium* sp	0.00±0.00^{i}	0.00±0.00^{i}	0.65±0.61^{gh}
	Cold Water	*Penicillium* sp	0.25±0.43^{hi}	1.07±0.43^{g}	2.50±0.80^{cd}
	Methanol	*Aspergillus niger*	0.00±0.00^{i}	0.18±0.32^{hi}	0.17±0.29^{hi}
	Methanol	*Fusarium* sp	0.45±0.00^{gh}	2.00±0.00^{de}	3.00±0.00^{bc}
	Methanol	*Penicillium* sp	2.50±0.00^{b}	2.70±0.00^{c}	3.00±0.00^{bc}
	Hot Water	*Aspergillus niger*	0.68±0.16^{fg}	1.28±0.41^{fg}	1.53±0.32^{ef}
	Hot Water	*Fusarium* sp	1.00±0.41^{de}	2.80±0.78^{bc}	2.95±0.67^{bc}
	Hot Water	*Penicillium* sp	0.00±0.00^{i}	0.00±0.00^{i}	0.28±0.26^{hi}
Acalypha winkensiana	Cold Water	*Aspergillus niger*	2.87±1.96^{b}	3.00±1.73^{b}	3.03±1.67^{bc}
	Cold Water	*Fusarium* sp	0.38±0.34^{hi}	0.58±0.52^{h}	1.05±0.93^{fg}
	Cold Water	*Penicillium* sp	0.22±0.19^{hi}	0.38±0.34^{hi}	0.70±0.17^{gh}
	Methanol	*Aspergillus niger*	1.35±0.00^{c}	2.05±0.00^{d}	2.35±0.00^{d}
	Methanol	*Fusarium* sp	0.45±0.00^{gh}	2.00±0.00^{de}	3.00±0.00^{bc}
	Methanol	*Penicillium* sp	2.75±0.00^{b}	3.18±0.00^{b}	3.48±0.00^{b}
	Hot Water	*Aspergillus niger*	0.75±0.00^{ef}	1.60±0.00^{ef}	1.80±0.00^{e}
	Hot Water	*Fusarium* sp	1.18±0.00^{cd}	2.28±0.00^{d}	2.58±0.00^{cd}
	Hot Water	*Penicillium* sp	3.92±0.14^{a}	4.00±0.00^{a}	4.00±0.00^{a}
Control	Methanol	*Aspergillus niger*	0.00±0.00^{i}	0.30±0.00^{hi}	1.10±0.00^{fg}
	Water	*Fusarium* sp	2.50±0.00^{b}	2.70±0.00^{c}	3.00±0.00^{bc}
	Methanol	*Penicillium* sp	0.10±0.00^{i}	0.00±0.00^{i}	0.95±0.00^{g}
	Water	*Aspergillus niger*	1.35±0.00^{c}	2.05±0.00^{d}	2.35±0.00^{d}
	Methanol	*Fusarium* sp	0.00±0.00^{i}	0.00±0.00^{i}	0.30±0.00^{hi}
	Water	*Penicillium* sp	0.45±0.00^{gh}	2.00±0.00^{de}	3.00±0.00^{bc}

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Table 3: The radial mycelia growth of the isolated fungal pathogens at 250mg/mL concentration of the plant extracts

Means with the same alphabets down the column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only

The radial mycelia growth of the isolated fungal pathogens at 500mg/mL concentration of the plant extracts (Table 4). At day 3, it was observed that cold water and hot water extracts of *Syzygium aromaticum* were effective in the reduction of the radial mycelia growth of *A. niger*, *Fusarium* sp. and *Pencillium* sp. (0.00 ± 0.00, 0.00 ± 0.00 and 0.00 ± 0.00) and (0.77 ± 0.10, 0.78 ± 0.00 and 0.00 ± 0.00) respectively as compared to the controls i.e *A.niger* (1.35 ± 0.00), *Fusarium* sp. (2.50 ± 0.00) and *Pencillium* sp. (0.45 ± 0.00).

At day 5, it was observed that cold water extracts of *S. aromaticum* was active in the reduction of radial mycelia growth of *A. niger* (0.48 ± 0.84), *Fusarium* sp. (0.00 ± 0.00) and *Pencillium* sp. (0.00 ± 0.00) respectively as compared to the controls i.e *A. niger* (2.50 ± 0.00), *Fusarium* sp. (2.70 ± 0.00) and *Pencillium* sp. (2.00 ± 0.00)

At day 7, it was observed that cold water extract of *S. aromaticum* had significant reduction on radial mycelia growth of *A. niger*, *Fusarium* sp. and *Pencillium* sp. (0.60 ± 1.40, 0.13 ± 0.23 and 0.00 ± 0.00) respectively as compared to the controls i.e *A.niger* (2.35 ± 0.00), *Fusarium* sp. (3.00 ± 0.00) and *Pencillium* sp. (3.00 ± 0.00) while hot water extract of the same plant was effective in the reduction of radial mycelia growth of *A. niger* (1.65 ± 0.00) and *Pencillium* sp. (0.00 ± 0.00) respectively as compared to the individual control. Furthermore, the methanol extract of *S. aromaticum* was effective only in the inhibition of the radial mycelial growth of *Aspergillus niger* (0.00±0.00) at P≤0.05.

Plant Extract	Solvent	Pathogens	Radial mycelia growth (cm) Day 3	Day 5	Day 7
Syzygium aromaticum	Cold Water	*Aspergillus niger*	0.00±0.00^{i}	0.48±0.84^{f}	0.60±1.04^{fg}
	Cold Water	*Fusarium* sp	0.00±0.00^{i}	0.00±0.00^{g}	0.13±0.23^{h}
	Cold Water	*Penicillium* sp	0.00±0.00^{i}	0.00±0.00^{g}	0.00±0.00^{h}
	Methanol	*Aspergillus niger*	0.00±0.00^{i}	0.00±0.00^{g}	0.00±0.00^{h}
	Methanol	*Fusarium* sp	0.45±0.00^{g}	2.00±0.00^{c}	3.00±0.00^{b}
	Methanol	*Penicillium* sp	2.50±0.00^{c}	2.70±0.00^{b}	3.00±0.00^{b}
	Hot Water	*Aspergillus niger*	0.77±0.10^{f}	0.95±0.15^{e}	1.65±0.05^{d}
	Hot Water	*Fusarium* sp	0.78±0.08^{f}	2.12±0.40^{c}	2.77±0.45^{bc}
	Hot Water	*Penicillium* sp	0.00±0.00^{i}	0.00±0.00^{g}	0.00±0.00^{h}
Acalypha winkensiana	Cold Water	*Aspergillus niger*	0.00±0.00^{i}	0.00±0.00^{g}	0.00±0.00^{h}
	Cold Water	*Fusarium* sp	0.18±0.32^{h}	0.53±0.68^{f}	0.62±0.82^{fg}
	Cold Water	*Penicillium* sp	0.00±0.00^{i}	0.13±0.23^{fg}	0.33±0.33^{gh}
	Methanol	*Aspergillus niger*	1.35±0.00^{d}	2.05±0.00^{c}	2.35±0.00^{c}
	Methanol	*Fusarium* sp	0.45±0.00^{g}	2.00±0.00^{c}	3.00±0.00^{b}
	Methanol	*Penicillium* sp	2.75±0.00^{b}	3.18±0.00^{a}	3.48±0.00^{a}
	Hot Water	*Aspergillus niger*	0.75±0.00^{f}	1.60±0.00^{d}	1.80±0.00^{d}
	Hot Water	*Fusarium* sp	1.18±0.00^{e}	2.28±0.00^{c}	2.58±0.00^{bc}
	Hot Water	*Penicillium* sp	2.95±0.05^{a}	3.35±0.05^{a}	3.47±0.15^{a}
Control	Methanol	*Aspergillus niger*	0.00±0.00^{i}	0.30±0.00^{fg}	1.10±0.00^{e}
	Water	*Fusarium* sp	2.50±0.00^{c}	2.70±0.00^{b}	3.00±0.00^{b}
	Methanol	*Penicillium* sp	0.10±0.00^{h}	0.00±0.00^{g}	0.95±0.00^{ef}
	Water	*Aspergillus niger*	1.35±0.00^{d}	2.05±0.00^{c}	2.35±0.00^{c}
	Methanol	*Fusarium* sp	0.00±0.00^{i}	0.00±0.00^{g}	0.30±0.00^{gh}
	Water	*Penicillium* sp	0.45±0.00^{g}	2.00±0.00^{c}	3.00±0.00^{b}

Table 4: The radial mycelia growth of the isolated fungal pathogens at 500mg/mL concentration of the plant extracts

Means with the same alphabets down the column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" on

The radial mycelia growth of the isolated fungal pathogens at 750mg/mL concentration of the plant extracts (Table 5). At day 3, it was observed that cold and hot water extracts of *Syzygium aromaticum* were effective in the control of all the fungal pathogens investigated for this experiment *Fusarium* sp. (0.00±0.00), *Penicillium* sp. (0.00±0.00) and *Aspergillus niger* (0.00±0.00) respectively when compared to the controls *Fusarium* sp. (2.50±0.00), *Penicillium* sp (0.45±0.00) and *Aspergillus niger* (1.35±0.00). It was further observed that cold water extract of *Acalypha wilkensiana* was also active in the reduction of the radial mycelia growth of all the fungal pathogens (*Fusarium* sp. (0.00±0.00), *Penicillium* sp. (0.00±0.00) and *Aspergillus niger* (0.00±0.00) respectively as compared to the individual control while the hot water extract of the same plant was potent in the reduction of radial mycelia growth of *Fusarium* sp. (1.68 ± 0.00) and *Pencillium* sp. (2.63 ± 0.00) respectively as compared to the control.

It was observed at day 5 that the cold water extract of *S. aromaticum*, and cold water extract of *A. wilkensiana* was effective in the control of all the fungal pathogens; *Fusarium* sp. (0.00±0.00), *Penicillium* sp. (0.00±0.00) and *Aspergillus niger* (0.00±0.00) respectively as compared to the controls i.e *Fusarium* sp. (2.70 ± 0.00), *Pencillium* sp. (2.00 ± 0.00) and *A. niger* (2.05 ± 0.00) while hot water extract of *S. aromaticum* was effective in the control of all the fungal pathogens (*Fusarium* sp. (0.00±0.00), *Penicillium* sp. (0.00±0.00) and *Aspergillus niger* (0.00±0.00) respectively as compared to the individual control. Hot water extract of *A. wilkensiana* was active in the reduction of radial mycelia growth of *Fusarium* sp. only. Similar observation was made at day 7 but hot water extract of *A. wilkensiana* was effective in the reduction of *Fusarium* sp. and *Pencillium* sp. only.

Plant Extract	Solvent	Pathogens	Radial mycelia growth (cm) Day 3	Day 5	Day 7
Syzygium aromaticum	Cold Water	*Aspergillus* niger	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{f}
		Fusarium sp	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{f}
		Penicillium sp	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{f}

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	Methanol	*Aspergillus niger*	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{f}
		Fusarium sp	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{f}
		Penicillium sp	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{f}
	Hot Water	*Aspergillus niger*	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{f}
		Fusarium sp	0.00±0.00^{f}	0.00±0.00^{e}	0.10±0.17^{f}
		Penicillium sp	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{f}
	Cold Water	*Aspergillus niger*	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{f}
		Fusarium sp	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{f}
		Penicillium sp	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{f}
	Methanol	*Aspergillus niger*	0.00±0.00^{f}	0.10±0.17^{e}	0.32±0.33^{f}
Acalypha winkensiana		*Fusarium* sp	0.00±0.00^{f}	0.00±0.00^{e}	0.27±0.25^{f}
		Penicillium sp	0.00±0.00^{f}	0.00±0.00^{e}	0.10±0.17^{f}
	Hot Water	*Aspergillus niger*	3.12±0.37^{a}	3.40±0.33^{a}	3.53±0.28^{a}
		Fusarium sp	0.37±0.38^{e}	1.29±0.19^{d}	1.68±0.18^{d}
		Penicillium sp	0.85±0.45^{d}	1.83±0.67^{c}	2.63±0.64^{c}
	Methanol	*Aspergillus niger*	0.00±0.00^{f}	0.30±0.00^{e}	1.10±0.00^{e}
	Water	*Fusarium* sp	2.50±0.00^{b}	2.70±0.00^{b}	3.00±0.00^{b}
	Methanol	*Penicillium* sp	0.10±0.00^{f}	0.00±0.00^{e}	0.95±0.00^{e}
Control	Water	*Aspergillus niger*	1.35±0.00^{c}	2.05±0.00^{c}	2.35±0.00^{c}
	Methanol	*Fusarium* sp	0.00±0.00^{f}	0.00±0.00^{e}	0.30±0.00^{f}
	Water	*Penicillium* sp	0.45±0.00^{e}	2.00±0.00^{c}	3.00±0.00^{b}

Table 5: The radial mycelia growth of the isolated fungal pathogens at 750mg/mL concentration of the plant extracts

Means with the same alphabets down the column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only

The *In-vivo* assessment of the effects of extracts of *Syzygium aromaticum* and *Acalypha wilkensiana* on the radial mycelial growth of the isolated fungal pathogens (Table 6). At 100mg/mL,, methanol, cold and hot water extracts of *S. aromaticum* had significant reduction on radial mycelial growth of A. niger (3.88 ± 0.88, 2.25 ± 0.00 and 2.85 ± 0.70) as compared to the control (5.13 ± 0.18, 5.48 ± 0.32). It was also observed that methanol, cold and hot water extracts of *A. wilkensiana* had significant reduction on radial mycelia growth of *A. niger* (2.58 ± 0.11, 1.48 ± 0.11 and 1.83 ± 0.11) as compared to the control (5.13 ± 0.18, 5.48 ± 0.32). Also, cold and hot water extract of the same plant also reduced radial mycelial growth of *Pencillium* sp. (1.53 ± 0.04 and 1.25 ± 0.00) as compared to the control (5.75 ± 0.35).

At .250mg/mL, cold and hot water of *S. aromaticum* had significant reduction on *Fusarium* sp. (2.30 ± 0.07 and 2.25 ± 0.71) and *Pencillium* sp. (2.20 ± 0.64 and 2.30 ± 0.28) as compared to the individual control (3.90 ± 0.78 and 5.75 ± 0.35). Methanol, cold and hot water extracts of *A. wilkensiana* at the same concentration significantly reduced *A. niger* (2.65 ± 0.21, 1.00 ± 0.14 and 1.75 ± 0.00), *Fusarium* sp. (1.20± 0.07, 1.50 ± 0.21 and 1.33 ± 0.25) and *Pencillium* sp. (1.65 ± 0.14, 1.85 ± 0.18 and 1.55 ± 0.28) as compared to the individual control i.e *A. niger*(5.13 ± 0.18 and 5.48 ± 0.32), *Fusarium* sp. (3.88 ± 0.67 and 3. 90 ± 0.78) and *Pencillium* sp. (3.25 ± 1.41 and 5.75 ± 0.35),

At 500mg/mL, cold and hot water extracts of *S. aromaticum* significantly reduced *A. niger* (1.75 ± 0.00 and 1.73 ± 0.11) and *Fusarium* sp. (1.88 ± 0.53 and 1.65 ± 0.14) as compared to the individual controls i.e *A. niger* (5.48 ± 0.32) and *Fusarium* sp. (3.90 ± 0.78) . Methanol, cold and hot water extracts of *A. wilkensiana* had significant reduction on radial mycelia growth of *A. niger* (1.98 ± 0.18, 0.65 ± 0.00 and 1.65 ± 0.18), *Fusarium* sp. (0.88 ± 0.11, 0.95 ± 0.42 and 1.28 ± 0.04 and *Pencillium* sp. (1.13 ± 0.18, 2.78 ± 0.6 and 1.50 ± 0.00) as compared to the individual controls i.e *A. niger* (5.13 ± 0.18 and 5.48 ± 0.32), *Fusarium* sp. (3.88 ± 0.67 and 3.90 ± 0.78) and *Pencillium* sp. (3.25 ± 1.41 and 5.75 ± 0.35 in the same concentration

At 750mg/mL. It was observed that methanol, cold and hot water extracts of *S. aromaticum* was effective in the reduction of *A. niger* (1.60 ± 0.14, 1.53 ± 0.18 and 2.20 ± 0.35), *Fusarium* sp. (2.53 ± 0.53, 1.83 ± 0.11 and 1.43 ± 0.46) and *Pencillium* sp. (1.70 ± 0.07, 2.00± 0.71 and 2.00 ± 0.35) as compared to the individual controls i.e *A. niger* (5.13 ± 0.18 and 5.48 ± 0.32), *Fusarium* sp. 3.88 ± .67 and 3.90± 0.78) and *Pencillium* sp. (3.25 ±1.41 and 5.75 ± .0.35) while methanol, hot and cold water extract of *A. wilkensiana* were significantly active on *A. niger* (1.48 ± 0.25, 0.53 ± 0.18 and 1.65 ± 0.14), *Fusarium* sp. (1.73 ± 0.25, 0.85 ± 0.57 and 1.65 ± 0.71) and *Pencillium* sp. (1.13 ± 0.04, 2.13 ± 0.18 and 1.49 ± 0.86) as compared to the individual controls i.e *A. niger* (5.13 ± 0.18 and 5.48 ± 0.32), *Fusarium* sp. 3.88 ± .67 and 3.90± 0.78) and *Pencillium* sp. (3.25 ±1.41 and 5.75 ± .0.35).

Plant	Pathogen	Solvent	Radial mycelia growth (cm) 100mg/mL	250mg/mL	500mg/mL	750mg/mL
Syzygium aromaticum	*A.niger*	Methanol	3.38±0.88^{de}	2.13±0.18^{d-i}	3.70±0.99^{bc}	1.60±0.14^{f-i}
		Cold	2.25±0.00^{d-i}	1.48±0.32^{f-i}	1.75±0.00^{g-k}	1.53±0.18^{f-i}
		Hot	2.85±0.07^{d-h}	2.50±0.35^{d-g}	1.73±0.11^{g-k}	2.25±0.35^{c-g}
	Fusarium sp	Methanol	3.63±1.24^{cd}	3.95±0.42^{bc}	3.00±0.00^{b-f}	2.53±0.53^{c-f}
		Cold	2.45±0.07^{d-i}	2.30±0.07^{d-h}	1.88±0.53^{g-j}	1.83±0.11^{fgh}
		Hot	2.38±0.25^{d-i}	2.25±0.71^{d-i}	1.65±0.14^{h-k}	1.43±0.46^{f-i}
	Pencillium sp	Methanol	5.25±1.41^{ab}	5.00±1.41^{ab}	1.98±0.32^{e-j}	1.70±0.07^{f-i}
		Cold	2.70±0.28^{d-h}	2.20±0.64^{d-i}	1.95±0.28^{f-j}	2.00±0.71^{e-h}
		Hot	1.65±0.21^{f-i}	2.30±0.28^{d-h}	2.05±0.28^{e-i}	2.00±0.35^{e-h}
Acalypha wilkensiana	*A.niger*	Methanol	2.58±0.11^{d-h}	2.65±0.21^{def}	1.98±0.18^{e-j}	1.48±0.25^{f-i}
		Cold	1.48±0.11^{g-i}	1.00±0.14^{i}	0.65±0.00^{k}	0.53±0.18^{i}
		Hot	1.83±0.11^{e-i}	1.75±0.00^{f-i}	1.63±0.18^{h-k}	1.65±0.14^{f-i}
	Fusarium sp	Methanol	1.85±0.28^{e-i}	1.20±0.07^{hi}	0.88±0.11^{jk}	1.73±0.25^{fgh}
		Cold	2.88±0.88^{d-h}	1.50±0.21^{f-i}	0.95±0.42^{ijk}	0.85±0.57^{hi}
		Hot	0.80±0.07^{i}	1.33±0.25^{ghi}	1.28±0.04^{ijk}	1.65±0.71^{f-i}
	Pencillium sp	Methanol	3.00±1.98^{d-g}	1.65±0.14^{f-i}	1.13±0.18^{ijk}	1.13±0.04^{ghi}
		Cold	1.53±0.04^{ghi}	1.88±0.18^{e-i}	2.78±0.60^{c-g}	2.13±0.18^{d-g}
		Hot	1.25±0.00^{hi}	1.15±0.28^{hi}	1.50±0.00^{h-k}	1.49±0.86^{f-i}
Control	*A.niger*	None	3.05±0.42^{d-g}	3.05±0.42^{cde}	3.05±0.42^{b-e}	3.05±0.42^{b-e}
		Water	5.48±0.32^{a}	5.48±0.32^{a}	5.48±0.32^{a}	5.48±0.32^{a}
		Methanol	5.13±0.18^{abc}	5.13±0.18^{a}	5.13±0.18^{a}	5.13±0.18^{a}
	Fusarium sp	None	3.35±0.14^{de}	3.35±0.14^{cd}	3.35±0.14^{bcd}	3.35±0.14^{bc}
		Water	3.90±0.78^{bcd}	3.90±0.78^{bc}	3.90±0.78^{b}	3.90±0.78^{b}
		Methanol	3.88±0.67^{bcd}	3.88±0.67^{bc}	3.88±0.67^{b}	3.88±0.67^{b}
	Pencillium sp	None	2.53±0.39^{d-h}	2.53±0.39^{d-g}	2.53±0.39^{d-h}	2.53±0.39^{c-f}
		Water	5.75±0.35^{a}	5.75±0.35^{a}	5.75±0.35^{a}	5.75±0.35^{a}
		Methanol	3.25±1.41^{def}	3.25±1.41^{cd}	3.25±1.41^{bcd}	3.25±1.41^{bcd}

Table 6: The radial mycelia growth of the isolated fungal pathogens at 100mg/mL, 250mg/mL, 500mg/mL and 750mg/mL concentrations of the plant extracts.

Means with the same alphabets down the column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only

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IV. DISCUSSION

In this study, the isolated pathogenic fungi from diseased pineapple were *Aspergillus niger, Pencillium* sp. and *Fusarium* sp. suggesting that these fungi isolates were responsible for fruitlet core rot disease of pineapple. Similar organisms have been isolated from pineapple, pawpaw, banana, orange and carrots as reported by Ewekeye *et al.* (2014); Abdullah *et al*. (2006) and Jacobs *et al.* (2010). These pathogens may have infected the fruit from the field with latent symptoms but cause severe damage under favourable environmental conditions in storage (Jay, 2003).

Pathogenicity test results proved that *Aspergillus niger, Pencillium* sp. and *Fusarium* sp. induced the characteristic symptoms of the original disease when re-inoculated on healthy pineapple fruits of the same species. It was found that *Fusarium* sp was the most virulent followed by *Pencillium* sp. while *Aspergillus niger* was the least virulent, which is in agreement with the work of Ghanbarzadeha *et al.* (2013) who reported that of all the fungal causal agents of basal rot of onion bulb, '*Fusarium oxysporum* and *Fusarium proliferatum'* were the most virulent strains. Brylinska and Siiwka (2016) also reported similar results where *Fusarium saimbucinum* was the most virulent strain of all the fungi species that were the causal agents of dry rot of potato. Lim (1985) also reported that both *Fusarium moniliforme* and *Penicillum funiculosum* were the causal agents of fruitlet core rot of pineapple but concluded that *F. moniliforme* was significantly less pathogenic than *P. funiculosum* contrary to what was obtained in this research.

In the *in-vitro* experiments, it was found that both *Syzygium aromaticum* and *Acalypha wilkensiana* at 100mg/mL, 250mg/mL, 500mg/mL and 750mg/mL had significant effects on the radial growth of the pathogens, however, hot and cold water extracts of *Syzygium aromaticum* and *Acalypha wilkensiana* had more inhibitory action against the pathogens as compared to methanolic extracts. The antimicrobial activity of plant extracts have been linked by many researchers to be due to the presence of phytochemical in them (Sofowora, 1993; Cowan, 1999). The effectiveness of the plant materials may be due to the solubility of the active compounds in water. Aqueous extracts of *Syzygium aromaticum* have been shown to have very high phenolic content and strong antioxidant properties by many researches conducted by different researchers (Duodonne *et al*., 2009; Kim et al. 2011 and Gülçin 2011). The highest activity of aqueous extracts in this study may be due to the resultant effect of eugenol and other minor compounds that also have antifungal activity. This indicated that aqueous extract contain compound that work synergistically with each other. This study was in agreement with the work of Avasthi *et al*. (2010) who reported that *Syzygium aromaticum* showed 100% inhibition on the mycelia growth of *Aspergillus niger* out of eight plant materials used. Similarly, it was in agreement with the study of Silva *et al*. (2014) who reported that aqueous extracts from *Syzygium aromaticum* are effective against a myriad of coffee fungal pathogens including those in the genus *Fusarium*. Aqueous extracts of the leaves of *A. wilkesiana* have also been confirmed to possess antimicrobial activities (Samuel and Afolabi, 2012; Cimanga *et al*., 2014) especially against *Aspergillus* sp.

In-vivo experiments were carried out with water and methanol extracts of *S. aromaticum* and *A.wilkensiana* at concentrations 10 folds higher than the *in-vitro* screening. The difference in concentration between the former and the latter is justified by the fact that there was little or no control over the environmental factors in the fruit such as diffusion of the extracts and the possibility that some of the tissues could even absorb the extracts. Hence the need to apply a higher concentration to be able to counter balance the factors mentioned above (Odebode and Che, 2001). In this study, the activity of water extracts of both medicinal plants had significant inhibitory action on the fungal pathogens at all concentrations. However, it could be suggested that the ability of *A. wilkensiana* to inhibit better in *in-vivo* screening maybe due to the increase in the volume of the extract at all concentrations.

In conclusion, the results of the present investigation indicates that both medicinal plant are potent in the inhibition of the metabolic activities of fungal pathogens that lead to spoilage and thus, they could be exploited as natural fungitoxicant to control the growth of pathogenic fungi and thus a better replacement of harmful synthetic chemicals (fungicides) which are commonly used for the control of rot thereby preserving the fruits nationwide**.**

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