African Multidisciplinary
Journal of Sciences and Artificial Intelligence

ISSN : 3434-9871

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AMJSAI.v1i1.3548

In Vitro Evaluation of Water and Ethanol Leaf Extracts of *Moringa Oleifera Azadirachta Indica* and *Carica Payaya* Against the Growth of Postharvest Fungal Pathogens of Pineapple (*Ananas comosus* (L) Merr.)

Amadioha A.C. & Anidobu R. J.
[1,2]Michael Okpara University of Agriculture, Abia State Nigeria
amadioha4u@yahoo.com

Article Info:

Submitted:	Revised:	Accepted:	Published:
Jun 26, 2024	Jul 12, 2024	Jul 26, 2024	Jul 31, 2024

Abstract

Fungi are primary infectious agents of agricultural products that cause significant economic losses of stored food products in Nigeria. Natural plant materials as alternative to synthetic pesticides are eco-friendly, readily available, biodegradable and cost effective. Pathogenicity test of fungal isolates from infected pineapple showed *Rhizopus stolonifer, Aspergillus niger* and *Fusarium solani* as pathogenic organisms causing postharvest deterioration of pineapple fruits. The *in vitro* evaluation of water and ethanol leaf extracts of *Moringa oleifera*, *Azadirachta indica* (Neem), and *Carica payaya* (pawpaw) was carried out to determine the antimicrobial potential of the plant extracts against the spore germination and mycelial radial growth of *R. stolonifer, A. niger* and *F. solani* causing rot disease of pineapple in storage. The ethanol and water leaf extracts of *A. indica*, *M. oleifera* and *C. papaya* evaluated as antifungal agents against the three pathogenic fungi *in vitro* exhibited varying levels of fungitoxicity on the spore germination and mycelia radial growth of the pathogens in culture. The inhibition of spore germination of the pathogens was in a dose dependent manner with 100 % concentration of Neem leaf extract being more potent in

inhibiting the spore germination of the pathogens followed by pawpaw and *Moringa* leaf extracts. The effect of the plant extracts on the mycelia radial growth inhibition of the pathogenic organisms in culture was also concentration dependent with the extracts being more effective from 40% concentration across the test plant materials. However, ethanol leaf extracts were more effective than water extracts in inhibiting the spore germination and mycelia radial growth of the pathogenic organisms in culture indicating that the solvent of extraction affected the fungitoxic activities of extracts of the plant materials with ethanol extracting more active compounds than water as extracting solvent. The antifungal potentials of the test plant materials could be exploited as biopesticide of plant origin in the control of postharvest microbial deterioration of pineapple and sustain the nutritional and market values.

Keywords: Water, Ethanol, Pineapple, Pathogens, *In Vitro*, *Moringa oleifera*, *Azadirachta indica*, *Carica payaya*

INTRODUCTION

Pineapple (*Ananas comosus* (L) Merr.) is a perennial monocotyledonous herb with 2,000 species in the Bromeliaceae family (Coppens and Leal, 2003). It is a popular nutritious fruit that grows in tropical and subtropical countries and one of the most profitable crops with biggest international market potential (Rhoda, 2008; Quijandria *et al.,* 2012; All Africa, 2013). It is an important source of income for smallholders that account for more than 70% of total production (Mark, 2010; McCulloch and Ota, 2002; Minot and Ngigi, 2003; Ssemwanga, 2007). The fruit has excellent juiciness and a source of vitamins and minerals and other immense health advantages including 80 to 89.2% moisture, 3-19% total solids with primary components of sucrose, glucose and fructose (Joy, 2010; Hossain *et al.,* 2015; Debnath *et al.,* 2012).

Despite its importance, pineapple is susceptible to a number of postharvest diseases which have been controlled with synthetic chemicals (Sago *et al.,* 2003). The structure and chemistry of pineapple fruits are affected by physical and chemical treatments (Fallik, 2004). Biological control of pathogenic organisms has been suggested as an effective and non-hazardous strategy to control major postharvest decays of pineapple fruits to improve crop production (Dalal and Kulkarni, 2013). The use of plant extracts in plant disease control not only aid in the reduction of inocula density or disease-producing activities of a pathogen, but play important role in sustainable agriculture and management of plant

pathogenic organisms (Junaid *et al.,* 2013). Many rain forest higher plants have been tested for fungicidal characteristics and have conclusively demonstrated to possess plant-based fungi-toxic characteristics for the treatment of disorders caused by fungi (Amadioha, 2001; 2003; 2004; 2012). These extracts of plant origin have the advantages of being readily available, biodegradable, inexpensive, environmentally friendly, less hazardous to non-target organisms, and used in Integrated Disease Management (IDM) programs by smallholder and resource-poor farmers (Amadioha, 2004). They are also found to give sustainable disease management options notably in organic farming where synthetic pesticides are not tolerated (Enyiukwu and Awurum, 2013).

Moringa oleifera Lam. (Plate 1) is a tropical deciduous perennial dicotyledonous tree with pale green and bipinnate or usually, tripinnate leaves used in the management of crop diseases due to its various bioactive ingredients that act in different ways against the pathogenic infection of plants (Holetz *et al.,* 2002; Leone *et al.,* 2015; Pandey *et al.,* 2011). Hussain *et al.* (2014) tested the effect of two different aqueous concentrations of *M. oleifera* leaf and seed extracts on the mycelial growth of two soil-borne diseases, *Fusarium solani* and *Rhizoctonia solani*, in an *in-vitro* investigation. At a concentration of 30%, both *Moringa* leaf and seed extracts inhibited the development of *F. solani* by 50%. The maximum percentage growth inhibition against *R. solani* was 45% and 50%, respectively, with extracts at 25% and 30% concentration levels. They concluded that *Moringa* seed and leaf aqueous extracts have antifungal activities, which inhibited the growth of *F. solani* and *R. solani* effectively. Also, the extracts have been utilized successfully as a seed therapy against *Sclerotium rolfsii*, a causative agent of damping off and stem rot of cowpea (Hussain *et al.,* 2014). *Moringa* extracts have been shown to inhibit the growth of several pathogenic fungi, including root and necrotrophic pathogens such as *Fusarium oxysporum, F. solani, Rhizoctonia solani, Botrytis cinerea, Monilina laxa, Alternaria alternata,* and *Pythium aphanidermatum* (Chen, 2009; Viera *et al.,* 2010; Badawy *et al.*, 2005). A phytochemical examination of aqueous extracts of *Maringa* leaf and seed revealed the presence of flavonoids, alkaloids, tannins, glycosides, terpenoids, and phenolic chemicals (Goss *et al.,* 2017). The leaves have also been shown to contain antimicrobial fatty acids, crystalline alkaloids, proteins, niazirin, and glycosides (El-Mohamedy and Abdallah, 2014). According to Abd El-Khair and Haggag (2007), these chemicals can either have direct inhibitory effects on infections by exhibiting fungicidal or fungistatic capabilities, or they can contribute in the formation of favourable conditions for antagonistic bacteria against the pathogenic organism.

a)  b) 

Plate 1: *Moringa oleifera* (a) plant, (b) leaf

Azadirachta indica A. Juss (Neem) is of Meliaceae family with pinnate leaves and dark green leaflets (Plate 2). It has many uses and the most important being the use of neem products to control crop insect pests and diseases without any harmful effect on the environment (Biswas *et al.,* 2002). Leaf extracts of *A. indica* suppressed mycelial growth and spore germination of *Helminthosporium oryzae* and *Pycularia oryzae*, causing blast and brown spot disease of rice plants respectively (Amadioha, 2012; 2006). Shrivastava and Swarakar (2014) demonstrated the fungicidal effect of *A. indica* aqueous leaf extract against *A. altanata* infecting pear fruits, with 85% reduction of fruit rot *in vivo*. *In vitro*, neem ethanol extracts inhibited the growth of *A. brassicola* and *F. oxysporum*, *M. phaseolina, F. moniliforme, F. Solani,* and *Botryodiplodia theobromae*, *A. tenuis, A. flavus, C. lunata,* and *R. stolonifer* (Aboellil, 2007). Another study by Anjali *et al.,* (2013) found that neem cake aqueous extracts inhibited spore germination in three sporulating fungi, *C. lunata, H. pennisetti,* and *C. gloeosporioides f. sp. mangiferae*. Other researchers discovered that *A. indica* methanol and ethanol extracts suppressed the growth of *Aspergillus flavus*, *Alternaria solani*, and *Cladosporium* spp. (Shrivastava and Swarnkar, 2014; Jabeen *et al.,* 2013).

Plate 2: *Azadirachta indica* Plant

***Carica papaya* L. (Pawpaw)** (Plate 3) is a member of the Caricaceae family and its leaves, barks, roots, latex, fruit, flowers, and seeds are utilized to cure a variety of ailments (Jaiswal *et al.,* 2010). Phytochemical examination of the leaves revealed that they contain saponins, cardiac glycosides, and alkaloids (Ayoola and Adeyeye, 2010). Aqueous extracts of *C. papaya* leaf and seed demonstrated antifungal action against *Colletotrichum gloeosporioides* and inhibited the mycelia growth of *Aspergillus flavus in vitro*. In greenhouse testing in Southeast Nigeria, *C. papaya* leaf extracts inhibited the spore germination of *Colletotrichum destructivum* in culture and development and spread of anthracnose caused by *C. destructivum*, and the results were superior to a synthetic fungicide (Enyiukwu and Awurum, 2013).

Plate 3: *Carica papaya* plant

This paper presents *in vitro* evaluation of the effects of water and ethanol leaf extracts of *M. oleifera*, *A. indica* (Neem), and *C. payaya* (pawpaw) on spore germination and mycelial radial growth of *R. stolonifer, A. niger* and *F. solani* causing rot disease of pineapple in storage.

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, Potato Dextrose Agar (PDA), was prepared by adding 39g of PDA in 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 (40^{o}C) 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 then cut into pieces (3mm) and used to inoculate the solidified PDA culture medium in plates. The inoculated plates were incubated at room temperature (27+2^{o}C) and examined daily for fungal growth which were sub-cultured on a fresh PDA medium to obtained pure cultures of the fungal isolates (Amadioha, 2001). Pathogenicity test was carries out on the isolates using surface sterilized healthy (uninfected) pineapple fruits to confirm and identify the pathogenic organisms (Barnett and Hunter, 2006).

Extract Preparation

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 20g, 40g, 80g, and 100g each of the leaf paste separately in 100ml of sterile distilled water or ethanol as solvent to obtain 20%, 40%, 80%, and 100% concentrations of water or ethanol

leaf extracts of the plant materials. The mixtures were 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 Spore Germination and Mycelia Radial Growth of Pathogens

The effect of the plant extracts on spore germination and mycelia radial growth of the pathogen was determined using the modified poisoned food techniques of Amadioha and Opara (2012). Suspensions of 5-day old cultures of the rot causing organisms were prepared by using 3 discs (1mm diam) of the test fungus in 10 ml of sterile distilled water and then filtered through a filter paper. The filtrate was standardized using a haemocytometer and used as spore suspensions ($1x10^5$ spores/ml) of the pathogens,

Effect of extract on Spore Germination

Spore suspensions (0.05ml) of the fungi were each separately added to different concentrations (20%, 40%, 60%, 80%, 100%) of the plant extracts, mancozade (synthetic fungicide) and the control (water or ethanol alone) and then separately placed on 3 sterile slides and incubated at 27°C for 24h in a humid chamber. One drop (0.5ml) of lacto-phenol in cotton blue was added on the slides before observation under a microscope. 100 spores were observed at random under a low power (x10) of the compound microscope and the number that germinated and those not germinated were recorded for each treatment to determine the percentage spore germination inhibition. Number of germinated and ungerminated spores for each treatment/replicate was counted and percentage spore germination inhibition calculated following the method of Amadioha (2004).

$$\% \text{ inhibition of spore germination} = \frac{TS-GS}{TS} \times \frac{100}{1}$$

Where

TS = Total number of germinated and ungerminated spores counted in treated plates

GS = number of germinated spores counted in treated plates

Effect of Plant Extracts on Mycelia Radial Growth of Pathogens in culture

The mycelia radial growth inhibition of the extracts was determined according to Amadioha (2004). Each extract of the test plant materials (0.5ml) was dropped on a

solidified PDA medium and spread to form a thin film on the surface of the medium. A disc (3mm diameter) obtained from the colony edge of 5 day- old culture of each fungal isolate was separately placed at the center of the extract PDA culture medium. Control experiments contained PDA and sterile distilled water or ethanol (0.5ml) alone. All the inoculated plates were incubated at room temperature (27^0C) and mycelia radial growth was measured after the growth in the control experiment had reached the edge of the control plate. Fungitoxicity was determined in terms of percentage mycelia radial growth inhibited and calculated according to Amadioha (2004).

Mycelia radial growth inhibition$(\%) = \frac{DC-DT}{DC} \times \frac{100}{1}$

Where:

DC = average diameter of control experiment.

DT = average diameter of fungal colony with extract treatment.

Data Analysis

Complete Randomized Design (CRD) with three replicates was used. 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

Effect of plant extract on spore germination of pathogens in culture

The inhibitory activity of different concentrations of the plant extracts against the spore germination of *R. stolonifer*, *A. niger* and *F. solani* (Table 1) showed significant ($p \leq 0.05$) variation in potency in spore germination inhibition of the pathogens in culture. Different concentrations of plant extracts inhibited to varying degrees spore germination of *R. stolonifer*. *A. niger* and *F. solani*. The spore germination inhibition increased significantly ($p \leq 0.05$) with increase in extract concentration. In control experiment with water alone, there was apparent spore germination inhibition of 2.5%, 0.00% and 7.0% of *R. stolonifer, A. niger* and *F. solani* respectively which may be due to incomplete spore viability rather than inhibition since no extract treatment was administered. The synthetic fungicide (mancozade) recorded total inhibition (100%) of spore germination in *R. stonolifer* and *A. niger* but had a reduced potency of 87 % inhibition of *F. solani* spores. The effect of water

and ethanol extracts of the plant materials on spore germination of the pathogens varied with concentration, solvent of extraction and pathogenic organism. For *R. stolonifer,* at 20% and 100 % concentration of water leaf extract, *Moringa* recorded 3.50% and 48.50% spore germination inhibition whereas Neem had 3.00% and 56.50%, and pawpaw, 5.50 % and 61.50 % spore germination inhibition respectively. The inhibitory effects at 20% and 100 % concentration of ethanol leaf extracts of *Moringa*, Neem and paw-paw against *R. stolonifer* were in the range of 7.50 % to 78.00 %, 14.00 % to 90.00 % and 11.50 % to 90.50 % spore germination inhibition respectively. For *A. niger* and *F. solani*, 20% concentration water extracts of the plant materials were not active (0.0% inhibition). At 40% to 100% concentration, *Moringa*, Neem and Paw-paw water leaf extracts recorded 4.50% to 28.50 %, 6.50 % to 34.00 % and 5.50 % to 33.50 % spore germination inhibition respectively against *A. niger* whereas *F. solani* had 3.50 % to 22.50 %, 8.50% to 32.50 % and 11.00 % to 36.00 %, spore germination inhibition respectively. Ethanol extracts of the plant materials recorded increased potency against the pathogens. At 20% to 100% concentrations, *Moringa* leaf extracts recorded spore germination inhibition of 7.50 % to 78.00 %, 6.00 % to 56.50 % and 6.00 % to 44.50 % against *R. stolonifer*, *A. niger* and *F. solani* respectively whereas Neem leaf extracts recorded spore germination inhibition of 14.00 % to 90.00 %, 7.50 % to 81.50 % and 10.00 % to 51.50 % and paw-paw leaf extracts, 11.50 % to 90.50 %, 6.50 % to 78.50 % and 12.00 % to 66.50 % against *R. stolonifer*, *A. Niger* and *F. solani* respectively.

Table 1. Effect of plant extract concentration on spore germination of pathogens

	Inhibition of spore germination (%)					
Extract	***Rhizopus stolonifer***		***Aspergillus niger***		***Fusarium solani***	
Concentration	Water ext.	Ethanol ext.	Water ext.	Ethanol ext.	Water ext.	Ethanol ext.
Moringa oleferia	$3.50^{k}\pm0.71$	$7.50^{k}\pm0.71$	$0.00^{i}\pm0.00$	$6.00^{i}\pm1.41$	$0.00^{i}\pm0.00$	$6.00^{i}\pm1.41$
20%	$6.50^{j}\pm0.71$	$15.00^{i}\pm1.41$	$4.50^{h}\pm0.71$	$8.50^{ik}\pm0.71$	3.50 ± 0.71	$95.0^{ke}\pm0.71$
40 %	$17.50^{h}\pm0.71$	$32.00^{g}\pm1.41$	$11.00^{g}\pm0.00$	$21.50^{h}\pm0.71$	$9.00^{gh}\pm1.41$	$18.00^{h}\pm0.00$
60%	$35.00^{f}\pm0.00$	$59.00^{e}\pm1.41$	$19.00^{e}\pm1.41$	$36.00^{f}\pm1.41$	$16.00^{ef}\pm1.41$	$35.00^{e}\pm1.44$
80%	$48.50^{d}\pm0.17$	$78.00^{c}\pm1.41$	$28.50^{c}\pm0.71$	$56.50^{e}\pm0.71$	$22.50^{d}\pm5.12$	$44.50^{d}\pm4.95$
100%						
Azadirachta indica	$3.00^{k}\pm0.00$	$14.00^{i}\pm1.41$	$0.00^{i}\pm0.00$	$7.50^{kc}\pm0.71$	$0.00^{i}\pm0.00$	$10.00^{k}\pm0.00$
20%	$11.50^{i}\pm0.71$	$25.50^{h}\pm0.71$	$6.50^{h}\pm0.71$	$12.00^{i}\pm1.41$	$8.50^{gh}\pm0.71$	$14.00^{ij}\pm1.41$
40%	$22.00^{g}\pm0.00$	$45.00^{f}\pm0.00$	$15.00^{f}\pm1.41$	$26.5^{0g}\pm0.71$	$14.00^{f}\pm1.41$	$24.50^{g}\pm0.71$
60%	$42.00^{e}\pm1.41$	$69.00^{d}\pm0.00$	$22.50^{d}\pm2.12$	$65.00^{d}\pm0.00$	$22.00^{d}\pm1.41$	$38.50^{e}\pm0.71$
80%	$56.50^{c}\pm0.71$	$90.00^{b}\pm1.41$	$34.00^{b}\pm1.41$	$81.50^{b}\pm0.71$	$32.50^{e}\pm0.00$	$51.50^{e}\pm0.71$
100%						
Carica papaya	$5.50^{j}\pm0.71$	$11.50^{j}\pm0.71$	$0.00^{i}\pm0.00$	$6.00^{c}\pm0.00$	$0.00^{i}\pm0.00$	$12.00^{ik}\pm2.83$
20%	$13.00^{i}\pm1.41$	$25.50^{h}\pm1.41$	$5.50^{h}\pm0.71$	$10.50^{ij}\pm2.12$	$11.00^{g}\pm1.41$	$16.50^{hi}\pm2.12$
40%	$33.56^{f}\pm0.71$	$45.00^{f}\pm0.00$	$16.00^{f}\pm0.71$	$23.50^{h}\pm0.71$	$17.00^{e}\pm1.41$	$28.50^{f}\pm0.71$
60%	$48.00^{a}\pm0.00$	$69.50^{d}\pm0.71$	$20.50^{de}\pm2.12$	$65.00^{d}\pm1.41$	$23.50^{d}\pm0.71$	$48.50^{c}\pm0.71$
80%	$61.50^{b}\pm0.71$	$90.50^{b}\pm0.71$	$33.50^{b}\pm2.12$	$78.50^{c}\pm0.71$	$36.00^{b}\pm2.83$	$66.50^{b}\pm0.71$
100%						
Mancozade	$100.00^{a}\pm0.00$	$100.00^{a}\pm0.00$	$100.00^{a}\pm0.00$	$100.00^{a}\pm0.00$	$87.00^{a}\pm0.00$	$87.00^{a}\pm0.00$
Control	$2.50^{k}\pm0.71$	$15.00^{i}\pm1.41$	$0.00^{i}\pm0.00$	$8.50^{ik}\pm0.71$	$7.00^{h}\pm0.00$	$15.50^{hij}\pm2.12$

Values are means of 3 replicates in two separate experiments. Values with same superscript are not significantly different ($P \leq 0.05$)

Water and ethanol leaf extracts of papaya recorded significantly the highest spore germination inhibition of the pathogens followed by *A. indica* and M. *oleifera*. The extracts

were more effective in inhibiting the spore germination of *R. stolonifer* followed by *A. niger* and *F. solani*. Also, the ethanol extracts were more effective in inhibiting the germination of the pathogen spores than the aqueous or water extracts of the test plants. The synthetic fungicide was more effective in inhibiting the spore germination of the pathogens. It recorded 100% inhibition of the germinating spores of the pathogens except with *F. solani* where the percentage spore germination inhibition was 87.00 %. The effect of 100.00 % concentration of ethanol extracts of the plant materials and the synthetic fungicide on spore germination inhibition of the pathogens are shown in Fig. 1.

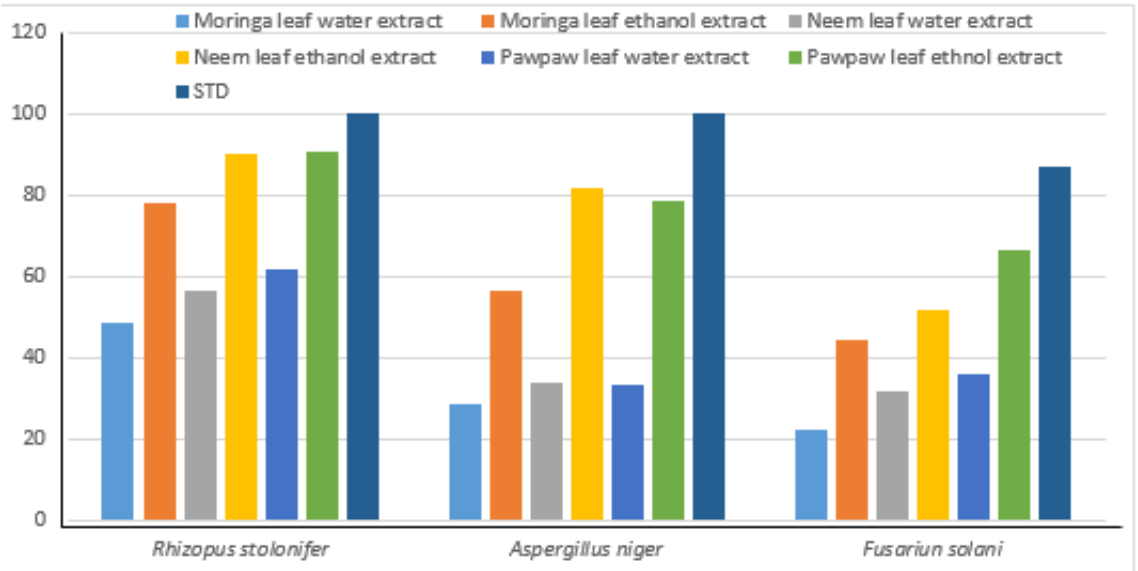

Fig. 1: Effect of ethanol leaf extracts (100% concentration) on spore germination of the pathogenic organisms in culture

Effect of plant extract on mycelia radial growth of pathogen in culture

The effect of different concentrations of plant extracts on mycelia radial growth of the pathogens in culture (Table 2) increased with concentration and varied according to solvent of extraction (water or ethanol). At 20 % to 100 % concentration of water leaf extract, the mycelia radial growth inhibition by *Moringa*, Neem and pawpaw on *R. stolonifer* was in the range of 17.53 % to 48.05 %, 20.78 % to 49.97 % and 21.43 % to 50.00 % respectively whereas ethanol extracts recorded, 24.66 % to 55.82 %, 27.91 % to 58.44 % and 25.69 % to 56.66 % respectively. The inhibition of mycelia radial growth by different plant extract concentrations against *A. niger* was similar to *R. stolonifer* but slightly lower in fungitoxicity. The water leaf extracts at 20 % to 100 % concentration caused reduction in mycelia radial

growth of *A. niger* to the tune of 15.67 % to 44.15 % (*Moringa*), 20.77 % to 41.55 % (Neem) and 22.07 % to 42.19 % (paw-paw) whereas the ethanol leaf extracts of *Moringa*, Neem and Pawpaw inhibited the mycelia radial growth of the fungus by 21.41 % to 48.72 %, 22.07 % to 49.97 % and 22.07 % to 50.64 % respectively.

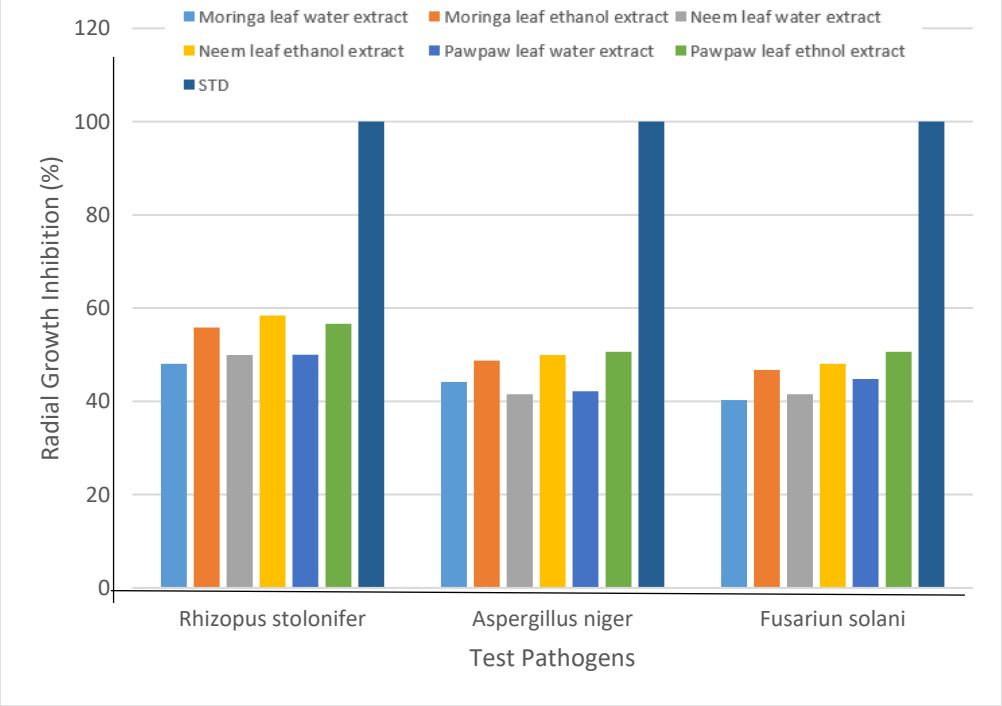

Fig. 2: Effect of 100% Concentration of Plant Extracts on Mycelia Radial Growth of the Pathogenic Organisms in Culture

Table 2:The Effect of Plant Extracts Concentration on Mycelia Radial

	Radial growth inhibition of pathogens (%)					
Extract concentration	***Rhizopus stolonifer***		***Asperigllus niger***		***Fusarium solani***	
	Water ext.	Ethanol ext.	Water ext.	Ethanol ext.	Water ext.	Ethanol ext.
Moringa oleferia						
20%	17.53^{g}±0.59	24.66 ±1.39	15.57^{c}±1.56	21.41^{j}±2.36	6.48^{i} ±1.72	22.07^{i} ±1.44
40%	21.39^{fg}±2.39	36.34^{f}±3.01	24.01^{ij}±2.31	32.48^{hi} ±1.27	18.17^{g} ±1.50	27.27^{ghi} ±1.34
60%	29.22^{e}±0.38	42.89^{de}±0.79	27.28^{gh}±0.50	38.94^{fg}±2.96	24.66^{f}±1.39	40.15^{cde}±12.12
80%	38.97^{ed}±0.71	47.39^{c}±1.89	33.76^{de}±1.22	43.46^{de}±1.99	33.75^{e}±3.05	38.97^{cde}±0.07
100%	48.05^{b}±0.96	55.82^{b}±2.65	44.15^{b}±1.03	48.72^{bc}±1.80	40.25^{ed}±1.10	46.75^{bc}±0.98
Azadirachta indica						
20%	20.78^{fg}±0.38	27.91±2.24	20.77^{k}±1.46	22.07^{j}±1.44	12.99^{h}±0.24	24.66^{hi}±1.39
40%	23.35^{f}±3.25	33.75^{f}±1.23	25.32^{hi}±0.45	33.12^{h}±0.30	20.11^{g}±2.39	31.82^{fgh}±0.33
60%	36.39^{d}±2.50	41.55^{e}±1.08	29.22^{fg}±0.38	40.25^{efg}±1.10	26.62^{f}±0.42	35.04^{efg}±3.03
80%	41.55^{e}±1.08	46.78^{c}±0.86	36.37^{cd}±0.67	46.12^{cd}±1.77	33.76^{e}±1.22	39.58^{ede}±3.87
100%	49.97^{b}±3.67	58.44^{b}±0.76	41.55^{b}±1.07	49.97^{b}±3.67	41.55^{c}±1.08	48.05^{b}±0.96
Carica papaya						
20%	21.43^{fg}±0.53	25.69^{jk}±1.44	22.07^{jk}±1.44	22.07^{jk}±1.44	20.77^{g}±1.46	27.27^{gki}±1.34
40%	23.35^{f}±3.25	34.42^{f}±0.42	29.23^{ghi}±0.42	29.23^{i}±0.38	24.02^{f}±0.48	32.46^{efgh}±1.24
60%	37.65^{cd}±1.15	40.25^{e}±1.00	31.16^{ef}±1.15	37.65^{g}±1.15	31.82^{e}±0.33	38.29^{def}±2.05
80%	40.92^{c}±0.16	44.15^{cd}±2.07	37.00^{c}±2.07	42.21^{ef}±0.14	37.65^{d}±1.15	44.81^{bcd}±0.96
100%	50.00^{b}±0.00	56.66^{b}±1.46	42.19^{b}±0.98	50.64^{b}±0.91	44.79^{b}±1.93	50.64^{b}±0.91
STD (Mancozade)	100%^{a}	100%^{a}	100%^{a}	100%^{a}	100%^{a}	100%^{a}

Values are means of 3 replicates in two separate experiments. Values with same superscript are not significantly different (P≤ 0.05)

The water leaf extracts of *Moringa*, Neem and pawpaw at 20 %-100 % concentration against *F. solani* recorded the range of 6.48 % to 40.25 %, 12.99 % to 41.55 % and 20.77 % to 44.79 % mycelia radial growth inhibition respectively whereas the ethanol leaf extracts were in the range of 22.07 % to 46.75 %, 24.66 % to 48.05 % and 27.00 % to 50.64 % respectively. The synthetic fungicide with 100.00 % mycelia radial growth inhibition and the plant extract at 100 % concentration that had the highest mycelia radial growth inhibition of the pathogenic organisms are presented in Fig. 2.

DISCUSSION

Pathogenicity test of fungal isolates from diseased pineapple fruits indicated *R. stolonifer, A. niger* and *F. solani* as the rot causing organisms of pineapple fruits in storage. 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 caused by *Thielaviopsis pradoxa* and soft or wet rots caused by *Ceratocystis paradoxa* (Paulin-Mahady *et. al.* (2002). Akinmusire (2011) reported *A. flavus, Rhizopus spp, Fusarium spp* and *Phytophthora spp* as major cause of losses of *Ananas comosus* in storage.

The leaf extracts of *Moringa*, Neem and pawpaw exhibited to varying degrees a dose dependent antifungal activities that inhibited the spore germination of the fungal pathogens suggesting that, spores of the pathogenic organisms showed varying levels of susceptibility to the different concentrations of plant extracts. The spores were more susceptible to ethanol extracts than water extracts indicating that the pathogens showed varying levels of susceptibility to fungitoxicity of different active principles of the plant materials in different solvent of extraction (Amadioha, 2006; 2012). The ethanol as an extracting solvent may have extracted more active ingredients than water that led to the recorded higher antifungal activities of ethanol extracts (Amadioha, 2003; Amadioha and Markson, 2007). Water and ethanol leaf extracts of papaya recorded the highest spore germination inhibition of the pathogens followed by *A. indica* and M. *oleifera* suggesting that papaya leaf extracts contained more active compounds than extracts of other plant materials.

The extracts were more effective in inhibiting the spore germination of *R. stolonifer* followed by *A. niger* and *F. solani* indicating that it was more susceptible to the extracts of the plant materials than other pathogenic organisms. The effect of the plant extracts on the mycelia radial growth inhibition of the pathogenic organisms in culture was also concentration

dependent with the extract being effective from 40% concentration across the extract of the plant materials. The antifungal effect of the plant extracts on the mycelia radial growth of the pathogens increased with concentration and varied according to solvent of extraction with ethanol extracts being more effective than water extracts indicating that the solvent of extraction affected the antimicrobial activities of the extracts (Amadioha, 2006; Amadioha, 2003; Amadioha and Markson, 2007).

Leaves of several plants have been used in the management of crop diseases due to the presence of various bioactive ingredients that act in different ways against the pathogenic infection of plants (Holetz *et al.,* 2002; Leone *et al.,* 2015; Pandey *et al.,* 2011). Hussain *et al.* (2014) tested the effect of two different aqueous concentrations of *M. oleifera* leaf on the mycelial growth of *Fusarium solani* and *Rhizoctonia solani*, in an *in-vitro* investigation. At a concentration of 30%, the leaf extracts inhibited the development of *F. solani* by 50%. The maximum percentage growth inhibition against *R. solani* was 45% with extracts at 25% concentration level and they concluded that *Moringa* leaf aqueous extracts have antifungal activities which inhibited the growth of *F. solani* and *R. solani* effectively. The extracts have also been utilized as a seed therapy against *Sclerotium rolfsii* causing damping off and stem rot of cowpea (Hussain *et al.,* 2014). *Moringa* extracts have been shown to inhibit the growth of several pathogenic fungi including root and necrotrophic pathogens such as *Fusarium oxysporum, F. solani, Rhizoctonia solani, Botrytis cinerea, Monilina laxa, Alternaria alternata,* and *Pythium aphanidermatum* (Chen, 2009; Viera *et al.,* 2010; Badawy *et al.*, 2005). *Azadirachta indica* (Neem) has been used to control crop diseases (Biswas *et al.,* 2002). Leaf extracts of *A. indica* suppressed mycelial growth and spore germination of *Helminthosporium oryzae* and *Pycularia oryzae*, causing blast and brown spot diseases of rice plants respectively (Amadioha, 2012; 2006). Shrivastava and Swarakar (2014) demonstrated the fungicidal effect of *A. indica* aqueous leaf extract against *A. altanata* infecting pear fruits *in vivo*. The ethanol extract of neem was evaluated *in vitro* against the growth of *A. brassicola, F. oxysporum*, *M. phaseolina, F. moniliforme, F. Solani,* and *Botryodiplodia theobromae*, *A. tenuis, A. flavus, C. lunata,* and *R. stolonifer* (Aboellil, 2007). Another study by Anjali *et al.,* (2013) found that neem aqueous extracts inhibited spore germination in three sporulating fungi, *C. lunata, H. pennisetti,* and *C. gloeosporioides f. sp. mangiferae*. *A. indica* methanol and ethanol extracts have been reported to suppress the growth of *Aspergillus flavus*, *Alternaria solani*, and *Cladosporium* spp. (Shrivastava and Swarnkar, 2014; Jabeen *et al.,* 2013). *In vitro* aqueous extracts of *C. papaya* leaves demonstrated antifungal action against *Colletotrichum gloeosporioides* and inhibited the

mycelia growth of *Aspergillus flavus*. Also, *C. papaya* leaf extracts inhibited the spore germination of *Colletotrichum destructivum* in culture and the results were superior to a synthetic fungicide (Enyiukwu and Awurum, 2013). The results of this study showed that extracts of the plant materials were significantly more effective than control experiments in spore germination inhibition and reduction of mycelial radial growth of the pathogenic organisms in culture indicating that extracts of the test plants contain antimicrobial activities that could be exploited as pesticides of plant origin in the control of postharvest microbial deterioration of pineapple fruits and increase food production in Nigeria.

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