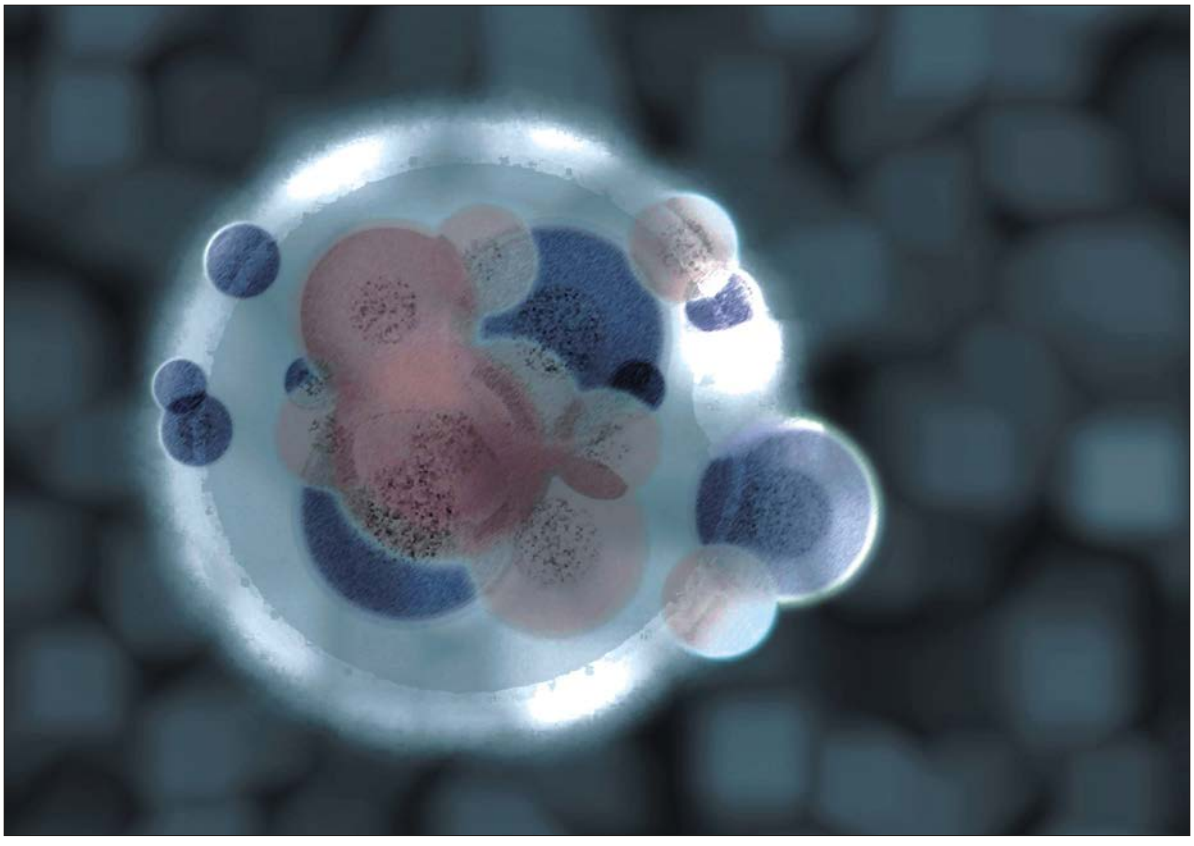

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 11 (5): 359-366, 2011
ISSN 1727-3048 / DOI: 10.3923/jbs.2011.359.366

Antimicrobial Activity of *Ficus* Leaf Extracts on Some Fungal and Bacterial Pathogens of *Dioscorea rotundata* from Southwest Nigeria

[1]O.A. Oyelana, [1]E.U. Durugbo, [2]O.D. Olukanni, [3]E.A. Ayodele,
[1]Z.O. Aikulola and [1]A.I. Adewole
[1]Department of Biological Sciences, Redeemer's University, Nigeria
[2]Department of Chemical Sciences, Redeemer's University, Nigeria
[3]Department of Botany and Microbiology, University of Ibadan, Nigeria

Abstract: Bio-control methods have become a common practice in horticulture and crop husbandry due to the attendant negative impact of fungicides and other chemical agents on soil, vegetation and environment. Most fungicides accumulate in plant tissues and subsequently trigger carcinogenic effects in animals and humans. The leaf extracts of *Ficus thonningii*, *F. saussureana*, *F. exasperata* and *F. sur* were screened for antimicrobial properties on eight (8) fungal species which included *Aspergillus flavus, A. niger*, *Botryodiploidia theobromae, Fusarium oxysporum*, *F. solani*, *Penicillium chrysogenum*, *P. oxalicum* and *Rhizopus stolonifer* and two (2) bacterial species viz; *Pseudomonas* spp. and *Klebsiella* spp. which were isolated from the rot portions of tubers of *Dioscorea rotundata*. The extracts from the *Ficus* species had low antimicrobial effect at 25 and 50 mg mL^{-1} concentrations while a significant arrest of mycelia growth was observed at 75 and 100 mg mL^{-1} concentrations. The presence of alkaloids, flavonoids and cardiac glycosides in the leaves of these species may have conferred the antimicrobial properties on these species. Application of the fungal pathogens isolated on healthy tubers and the subsequent development of rots confirmed these organisms as the natural pathogens of this crop. The extracts from all the four *Ficus* species exerted significant antimicrobial effect on all the test organisms at 75 and 100 mg mL^{-1} concentrations and its application at these concentrations would help to minimise infection and spoilage during and after storage and improve farmers' revenue.

Key words: *Dioscorea rotundata*, antimicrobial, *Ficus* species, bio-control, leaf extract, pathogenicity

INTRODUCTION

The cultivated yams belong to the genus *Dioscorea* with over 200 varieties (Norman *et al*., 1995; FAO, 2005) of which six (6) species are the most common foods in tropical countries across the West Africa, Caribbean and North Central of the South east Asia (Ajalie and Okigbo, 2005). The most important and widely cultivated member of this group is *Dioscorea rotundata* (White yam) (Asadu and Akamigbo, 1996). The 21, 814 million tons of yam produced in Nigeria annually (FAO, 1998; Ajalie and Okigbo, 2005) may be eaten boiled, fried or roasted or as yam pastes by the Yorubas and as ingredients for few confectionaries (Okaka *et al*., 1991).

Yams are valued food crops, a major source of carbohydrates, vitamins and dietary fibres (Ekefan *et al*., 1999; Ogaraku and Usman, 2008) and equally known to contain medicinal properties for the treatment of diabetes mellitus and hyperchosterolemia (Okigbo and Ogbonna, 2006). Postharvest losses account for about 40% of valuable yam stock (Awuah and Akrasi, 2007) and rots from microbial infestation of healthy tubers reduce their table quality and renders them unappealing to consumers (Amusa, 1999). Microbial infestation and the spread of rots are severe in the tropics where temperature and humidity are high. The fungal and bacterial pathogens implicated in the development and spread of rots are *Aspergillus flavus* ex Fr., *A. niger*, *A. tamari*, *Botryodiploidia theobromae*, *Cladosporium herbarium*, *C. sphaerospermum* and *Cylindrocarpon radiocola* while *Corynebacterium* spp. *Serratia* spp. and *Erwinia* spp are the representative bacteria involved in yam rot diseases (Okigbo, 2004).

The incidence of rot varies with the species of fungi and bacteria, the varieties of yam and the physical or health state of tubers. Infection is accentuated by a breakdown of tissues of the peridermal layer through wounds inflicted by rodents, nematodes and farm implements or improper handling of tubers (Ogaraku and Usman, 2008). The management and control of rot disease has been a major challenge to yam farmers the world over. The use of chemicals such as Sodium o-phenylphenate, Borax, Captan, Thiobendazole, Naphthalene acetic acid and Sodium hypochlorite have been quite effective.

Corresponding Author: O.A. Oyelana, Department of Biological Sciences, Redeemer's University, Nigeria

However, their uses have been associated with a number of deleterious and physiological effects on plant tissues (Amusa and Ayinla, 1997).

A number of plant extracts have been proven useful as biological control agents without side effects on both humans and environment (Omoseyindemi, 2003; Okwute, 1992). The extracts from few leaves including *Carica papaya*, *Cassia alata*, *Cassia fistula* and stems of *Citrus limon* inhibited the growth of *Collectotrichum gloeosporiodes* (Banos *et al*., 2002) and *Microsporum gypseum*, *Trichophyton rubrum* and *Penicillum marneffei* (Suleiman *et al*., 2008). Srivastava *et al*. (2011) observed a significant reduction in the growth of *Fusarium oxysporium* with 15% concentration of extracts from leaves of both Neem and Lantana plants. The application of wood ash and palm oil to cut surfaces of yam tubers prevented rot diseases caused by *Aspergillus niger*, *Penicillum chrysogenum* and *Rhizopus stolonifer* (Awuah and Akrasi, 2007). Ashraf and Khan (2007) reported effectiveness of the extracts from *Gliocladium virens* on the growth and multiplication of *Meloidogyne javanica* (nematode) infecting some eggplants. Equally, the extracts from leaves of *Zingiber officinale*, *Vernonia amygdalina*, *Senna alata*, *Cymbopogon citrates* and *Ricinus communis* are well documented antifungal agents against yam rot (Amusa, 2001; Nahunnaro, 2008).

The Nigerian forest is replete with over 45 different species (Sonibare *et al*., 2005) of the 800 members of the genus *Ficus* already characterised (Zerega *et al*., 2005). Members of this group can be found in savanna and rainforest ecosystems and beside rivers and streams. They are known for their medicinal properties and pharmacological values (Kubmarawa *et al*., 2007). Some phenolic compounds with pharmacological properties including funarocoumarins (psoralen and bergapten), flavonoids (rutin, phenolic acids,) and also phytosterol (Teixeira *et al*., 2006) were found to be abundant in this species. *Ficus carica* is rich in phenols, essential oils and flavonoids and other bioactive compounds such as arabinose, β-amyrins, β-carotenes, glycosides, β-setosterols and xanthotoxol (Aref *et al*., 2010).

The efficacy of *Ficus* extracts has not been documented on known pathogens of tuber crops, particularly among the members of *Dioscorea*. Consequently, liquid extracts from leaves of four (4) of the forty five (45) *Ficus* species in Nigeria were tested for their antimicrobial properties on isolated fungal and bacterial pathogens of *Dioscorea rotundata* with the view of providing a cheap and ready source of biological control to improve the market value of this very important tuber crop in Nigeria.

MATERIALS AND METHODS

Collection of yam tubers and plant materials: Yam tubers with symptoms of rots were collected along with fresh healthy ones from markets and distribution points in four towns in each of Ekiti and Lagos States, Nigeria in 2009 and 2010. A total of 160 yam tubers samples were collected for the study. These samples were sealed and transported in sterile polythene bags and later stored at room temperature in the laboratory.

The whole shoot and foliages of *Ficus thonningii* (Blume), *F. saussureana* (De Candolle), *F. exasperata* (Vahl) and *F. sur* (Forssk) were collected from the Biological garden of the Department of Botany and Microbiology, University of Ibadan, Nigeria and fields across the ancient city of Ibadan, Oyo State, Southwest Nigeria. The plants were air dried at room temperature and stored at 24°C until ready for use.

Preparation of culture media: The Potato Dextrose (PDA) and Mueller Hilton agar for the respective culturing of fungi and bacteria were prepared according to the manufacturer's instruction and following the techniques described by Arora and Arora (2008) for the purpose of obtaining pure cultures.

Isolation of fungal pathogens: The yam tubers with symptoms of rot were first rinsed with deionised water, surface sterilized for a minute with Sodium hypochlorite and air dried. The technique of isolating the fungal pathogens was as described by Willey *et al*. (2008).

Isolation of bacterial pathogens: The yam tubers with symptoms of rot were first rinsed with deionised water, cut open and pieces with a dimension of 3×3×2 mM of the infested portions were lifted. The pure culture of the test organisms was obtained using the techniques described by Arora and Arora (2008) on Mueller Hilton agar. The test was set up in triplicate.

Biochemical test analysis: The different biochemical tests analysis included Gram staining procedure as described by Cheesbrough (2002), citrate utilization test (MacWilliams, 2009), Hydrogen sulphide production test (Lehman, 2009) and Sugar fermentation and oxidase tests (Cowan and Steel, 2002). The isolates were later identified using Bergey's manual as described by Brenner *et al*. (2005).

Preparation and application of plant extracts: Cold extraction of the powdered plant materials was done with ethanol as organic solvent. Following the techniques of

Junaid *et al.* (2006), specific quantity of the powdered plant materials was soaked in the extraction solvent, filtered using Whatman No. 1 filter paper and filtrate was concentrated using rotary evaporator at 60°C.

Antimicrobial activity assay: The technique of Okigbo and Ogbonna (2006) was adopted for the screening of the plant extracts on the isolates with the 20 uL of the different concentrations (100, 75, 50 and 25 mg mL^{-1}) poured in the agar wells. Dimethylsulfoxide solvent and 10 μg mL^{-1} cefoxitin were respectively applied as negative and positive controls for both treatments. The diameters of zones of inhibition were measured (mM) using a ruler. The experiment was set up in triplicate.

Minimal Inhibitory Concentration (MIC): The determination of the minimum inhibitory concentration of the extracts was as described by Adeniyi and Ayepola (2008) using 12.5-50 mg mL^{-1} concentrations of the extracts. The extract concentration(s) around which there was least visible growth was regarded as the minimal inhibitory concentration.

Qualitative phytochemical analysis: Aqueous extracts of the four *Ficus* species were screened for the presence of bioactive chemicals including alkaloids using Mayer's reagents, tannins and saponins using ferric salt and frothing tests respectively in line with standard procedures described by Harborne (1998) and Parekh and Chanda (2007). Cardiac glycosides were determined by Keller-Keliani's tests while sodium hydroxide test was employed for the detection of flavonoids as described by Trease and Evans (2002). Two hundred milligrams of the extracts was dissolved in appropriate solvents: methanol for alkaloid determination and deionised water for both tannins and saponins.

Pathogenicity test: The yam tubers were prepared for Pathogenicity tests following the techniques described by Priou *et al.* (1999). The inoculated yam tubers were covered with the yam tissue 6 cm in diameter, sealed with a wax and left for six weeks.

Statistical analysis: Percentages and statistical mean were employed to analyse the data generated. The different effects of *Ficus* extracts on the test organisms was analysed using ANOVA.

RESULTS AND DISCUSSION

The fungal pathogens isolated from rot portions of the different yam tubers included *Aspergillus flavus*, *A. niger*, *Botryodiploidia theobromae*, *Fusarium oxysporium*, *Penicillium chrysogenum*, *P. oxalicum* and *Rhizopus stolonifer* (Table 1). A similar profile of fungal species was obtained from some yam tubers (Okigbo, 2004; Okigbo and Nmeka, 2005) in Southeast, Nigeria. However, *Mucor circinelliodes* and *Sclerotium rolfsii* were consisted in related group of fungal pathogens isolated from some yam tubers also from Southeast, Nigeria (Nahunnaro, 2008). *Fusarium solani* was the rare inclusion in the similar fungal profile isolated from yam tubers in Owerri, Imo State, Southeast, Nigeria. A similar fungal isolates from Yola town in Adamawa State, Northeast, Nigeria (Yusuf and Okusanya, 2008) and Keffi in Nassarawa State, North central, Nigeria (Ogaraku and Usman, 2008) consisted of *Sclerotium rolfsii* and *Rhizoctonia* spp. and *F. solani* as rare inclusions, respectively. These rare inclusions may be linked to the differences in soil and climatic conditions and as well as the prevailing and dominant fungi spores at harvest.

The percentage occurrence of *Fusarium oxysporium* and *Penicillium oxalicum* was 100% and they were found in all the plates examined (Table 1) while the percentage occurrence for *Fusarium solani* was 92 and 89% for both *Aspergillus niger* and *Penicillum chrysogenum*. Ezeibekwe *et al.* (2009) reported similar spread and observed that *Fusarium oxysporium* and *Botryodiplodia theobromae* occurred 33.3% more than other organisms.

Table 1: The percentage occurrence of the isolated bacterial and fungal pathogens in the sampled yam

		Occurrence			
Group	Isolated organism	1st (12) sets of plates	2nd (12) sets of plates	3rd (12) sets of plates	Average (%)
Bacteria	*Pseudomonas* spp.	4	2	6	33
	Klebsiella spp.	2	-	4	17
Fungi	*Aspergillus flavus*	12	8	10	83
	Aspergillus niger	12	8	12	89
	Botryodiploidia theobrome	11	9	10	83
	Fusarium oxysporium	12	12	12	100
	Fusarium solani	12	11	10	92
	Penicillium chrysogenum	10	11	11	89
	Penicillium oxalicum	12	12	12	100
	Rhizopus stolonifer	10	10	8	78

Table 2: Induced infection on yam tubers using the bacterial and fungal pathogens

Group	Isolated organism	1st set of yam (10) (Average) mM	2nd set of yam (8) (Average) mM	3rd set of yam (12) (Average) mM	Total average (%) mM
Bacteria	*Pseudomonas* spp.	4	0	0	1
	Klebsiella spp.	0	0	0	0
Fungi	*Aspergillus flavus*	48	57	61	55
	Aspergillus niger	46	59	54	53
	Botryodiploidia theobrome	47	59	56	54
	Fusarium oxysporium	50	57	57	55
	Fusarium solani	62	59	58	60
	Penicillium chrysogenum	64	60	61	62
	Penicillium oxalicum	57	57	62	59
	Rhizopus stolonifer	55	56	55	55

Table 3: Organisms and associated diseases

Group	Isolated organism	Associated diseases	Authourity
Bacteria	*Pseudomonas* spp.	Gram-ve enteric bacteria. *P. aureginosa* causes pneumonia, UTI, causes hospital acquired infections, meningitis and brain abscesses, ear infections, eye, bone and joint infections.	Todar (2011)
	Klebsiella spp.	*K. pneumonia* and *K. oxycot* resistant to ampicillin. Causes community acquired lobar pneumonia in alcoholic men above 40 yrs who have comorbid conditions like diabetes, chronic obstructive pulmonary disease.	Gasink *et al.* (2009) Khan *et al.* (2009)
Fungi	*Aspergillus flavus*	Pulmonary aspergillosis, post harvest disease of lemon fruits. A wound invading pathogen.	Mahgoub and El Hassan (1972) Kadowaki *et al.* (2007)
	Aspergillus niger	Causes allergic conditions, stuffy nose, pulmonary oxalosis, aspergillosis. Crown rot of groundnut.	Kierownik (1990)
	Botryodiploidia theobromae	Rots of yam tubers, leaf spot of mango. Storage rots of cocoyam.	Okigbo *et al.* (2010) Okigbo and Osuinde (2003) Ugwnanyi (2008), Okigbo and Ogbouna (2006)
	Fusarium oxysporium	Storage rots of cocoyam. Wilt disease of guava, lettuce and banana.	Ugwuanyi (2008) Gupta *et al.* (2010)
	F. solaui	Diseases of cucurbits, soybean. Lung diseases in man.	Amey *et al.* (1997) Mehl and Epstein (2007)
	Penicillium chrysogenum	Intestinal diseases, pneumonia.	Barcus *et al.* (2005)
	P. oxalicum	Tomato mold, ear rot of com.	Kwon *et al.* (2008)
	Rhizopus stolonifer	Soft rot of squash. Head rot of sunflower.	Kwon *et al.* (2000)

The *Pseudomonas* spp. and *Klebsiella* spp. are the two bacteria pathogens isolated from this study (Table 1). This result differs from the ones reported by Okigbo (2004) for the sampled yam tubers from the Southeast Nigeria and his results also tallied with the observations of Amusa (1999) who reported similar bacterial profile for some yam tubers from North central Nigeria. This is the first time a different bacteria profile is being implicated in the rot disease of yam tubers in the Southwest Nigeria. However, the Pathogenicity test could not confirm these bacteria as being majorly responsible for the rot disease (Table 2). They are likely opportunistic organisms found in association with the isolated fungi species.

The isolated fungal species were confirmed as the natural pathogens responsible for the rot disease in the sampled yam tubers (Table 2). The intrinsic ability of some isolated fungal pathogens at causing significant rot of exposed yam tubers has equally been reported (Okigbo and Ogbonna, 2006). The average spread of the rotted area (48 mM-64 mM) was observed for all the fungal species. *Penicillium chrysogenum* exhibited a wider rotted area (62 mM) followed by *Fusarium solani* with 60 mm spread (Table 2). A significantly different result was reported by Ezeibekwe *et al.* (2009) in which they observed that *Rhizopus oryzae* exhibited a 25.37 cm spread and a 17.20 cm and 12.65 cm spread by *Botryodiplodia theobromae* and *Fusarium solani*, respectively. These implicated organisms posed a significant threat to the revenue of farmers and the health of consumers at large.

The consequence of this contamination may be severe, as these fungi and bacteria have been implicated in a number of gastro-intestinal, respiratory and related health complications in humans (Table 3). The soil may have been contaminated by sewage and other domestic and household wastes. The samitary state of most storage facilities is deplorable and farm produce are often exposed to contamination while in storage. Regular fumigation and cleaning of these facilities will minimise the incidence of spoilage and ensure good market value for these products. There is paucity of data on bacteria induced yam rot, particularly in Nigeria and the volume of reports

Table 4: The antimicrobial effects of *Ficus* extracts on bacterial and fungal pathogens a, b, c, d = diameter of inhibition in millimeter

		Ficus thonningii (mg mL^{-1})				*Ficus exasperata* (mg mL^{-1})				*Ficus sur* (mg mL^{-1})				*Ficus saussureana* (mg mL^{-1})				
Group	Isolated organism	100a	75b	50c	25d	100a	75b	50c	25d	100a	75b	50c	25d	100a	75b	50c	25d	Positive control
Bacteria	*Pseudomonas* spp.	8	6	6	5	11	8	8	4	11	11	8	3	8	6	4	4	19
	Klebsiella spp.	8	8	4	4	11	10	6	4	9	8	11	4	8	6	6	4	18
Fungi	*Aspergillus flavus*	14	14	8	12	12	10	8	8	10	8	6	4	6	6	6	5	18
	Aspergillus niger	14	13	11	11	11	10	10	7	11	10	6	3	8	7	6	8	17
	Botryodiploidia theobrome	10	8	6	8	12	10	11	8	12	10	8	4	7	6	4	4	16
	Fusarium oxysporium	9	8	7	9	12	9	10	6	11	8	6	4	6	4	0	2	15
	Fusarium solani	12	8	10	8	14	10	8	8	12	10	8	3	8	8	6	4	18
	Penicillium chrysogenum	14	10	11	12	12	11	11	8	10	6	4	4	8	7	4	6	18
	Penicillium oxalicum	13	8	10	10	12	12	10	6	8	6	6	2	8	8	5	7	16
	Rhizopus stolonifer	14	10	10	13	14	12	10	7	10	8	6	4	9	6	3	5	18

50-100 mg mL^{-1} concentrations inhibited fungal growth ($p = 0.05$) significantly while growth of bacterial pathogens was successfully inhibited at 75-100 mg mL^{-1} when compared with positive controls

from local studies has been on fungal pathogens which are otherwise considered major pathogens that can affect the table and market values of tuber crops in the tropics. The combined analysis of variance (ANOVA) showed that the extracts from the four *Ficus* species exhibited a significant ($p = 0.05$) difference against the growth of all the Fungi species studied at 75-100% concentrations (Table 4). The minimum inhibitory concentration (2-12 mM) at 25 mg mL^{-1} was equally effective at interfering with the growth of all the fungal pathogens identified. The strong antimicrobial activity of these extracts confirms their efficacy as bio-control agents against the rot disease and the causative agents. There was no visible inhibition of fungal growth around the two wells designated as positive and negative controls.

Lopez *et al*. (2005) reported that *Ocimum* oil was active against several species of bacteria (*Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Shigella* and some *Salmonella* spp. and *Proteus* spp.) and fungi (*Trichophyton rubrum, T. mentagrophytes, Cryptococcus neoformans, Penicillium islandicum* and *Candida albicans*). Alhussaen *et al*. (2011) reported a significant reduction in the growth of *Pythium ultimum* on the foliages of tomato seedlings exposed to undiluted garlic extract. Extracts from foliages of some plants (Sharma *et al*., 2003; Nahunnaro, 2008; Kubmarawa *et al*., 2009) confirmed them as effective bio-control against few fungal pathogens of yam tubers. The popular *Azadirachta indica* proved efficacious on some fungal pathogens of yam tubers (Okigbo and Ogbonna, 2006). Boughalleb *et al*. (2010) reported resistance to *Fusarium* isolates by *Cucurbita* species grafted with *Citrullus lanatus* (watermelon). Plant extracts are relatively low in toxic substances and exert no deleterious and mutagenic effects on plants tissues (Kubmarawa *et al*., 2009) and hence, their use is gaining more importance among the local farmers.

The presence of alkaloids, flavonoids and cardiac glycosides in the four *Ficus* species screened may have conferred the antimicrobial properties on these groups of plants and which helped transform them as effective bio-control agents against the isolated bacterial pathogens. Flavonoids and phenolic compounds including psoralen and bergapten (Teixeira *et al*., 2006) and essential oils and other bioactive compounds such as arabinose, β-amyrins, β-carotenes and glycosides (Aref *et al*., 2010) have been found to be in abundant in some *Ficus* species. The essential oil of pepper fruit and activity of the phenolic acids have been shown (Ejechi *et al*., 1999) to be effective against tomato rot fungi. They suggested that the presence of b-phenylnitroethane may be responsible for the pungent properties of this essential oil.

Ejechi and Akpomedaye (2005) equally reported the efficacy of some essential oils against some food borne microorganisms including *Staphylococcus aureus, Salmonella* spp. *Pseudomonas aeruginosa, Proteus* spp. *Escherichia coli, Enterococcus faecalis, Serratia* spp. *Bacillus* spp. *Clostridium* spp. *Penicillium* spp. and *Aspergillus flavus* isolated from food products. The leaf extracts from the species of *Ficus* demonstrated a broad-spectrum of activity against *Pseudomonas* and *Klebsiella* spp and the fungal pathogens and which confirms them as antimicrobial agents for the control of rot diseases of yam tubers, particularly in the tropics and similar environment.

CONCLUSION

The profile of fungi and bacteria pathogens implicated in the yam rots may be dependent on the prevailing fungal spores at harvest and the severity of rot was accentuated by soil and prevailing climatic conditions. The contamination of soil through domestic and household wastes and poor sanitary condition of the storage facilities were suggested as sources of fungi infection. The fungal pathogens isolated included *Aspergillus A. niger, Botryodiploidia theobromae,*

Fusarium oxysporium, Penicillium chrysogenum, P. oxalicum and *Rhizopus stolonifer* and were confirmed as causative agents of yam rots in southwest, Nigeria. The bacteria isolated viz: *Pseudomonas* spp. and *Klebsiella* spp. were regarded as opportunistic organisms as they could not be confirmed as rot agents. The leaf extracts at 50 mg mL^{-1}-100 mg mL^{-1} concentrations had profound antimicrobial properties on the fungal and bacterial organisms. These extracts contained alkaloids, flavonoids and cardiac glycosides and may have conferred the antimicrobial properties on this group of plants.

ACKNOWLEDGMENT

The financial assistance and facilities for the successful completion of this study was provided by the Redeemer's University, Nigeria. Miss O. O. Fatoki typed the manuscript.

REFERENCES

Adeniyi, B.A. and O.O. Ayepola, 2008. The phytochemical screening and antimicrobial activity of leaf extracts of *Eucalyptus camaldulensis* and *Eucalyptus torelliana* (Myrtaceae). Res. J. Med. Plant, 2: 34-38.

Ajalie, A.N. and R.N. Okigbo, 2005. Inhibition of some human pathogens with tropical plants extracts *Chromolaena odorata*, *Citrus aurantifolia* and some antibiotics. Int. J. Mol. Med. Adv. Sci., 1: 34-40.

Alhussaen, K., E.I. Hussein, K.M. Al-Batayneh, M. Al-Khatib and W.A. Khateeb *et al*., 2011. Identification and controlling *Pythium* sp. infecting tomato seedlings cultivated in Jordon Valley using garlic extract. Asian J. Plant Pathol., 5: 84-92.

Amusa, N.A. and M.A. Ayinla, 1997. The effect of tecto (Thiabendazole) on the activities of yam rot causing fungi and on sprouting of yam set. Int. J. Trop. Plant, 14: 113-120.

Amusa, N.A., 1999. Concentric leaf spot of yam (*Dioscrorea* sp.) in South-western Nigeria. Mycopathologia, 148: 33-36.

Amusa, N.A., 2001. Screening of cassava and yam cultivars for resistance to anthracnose using toxic metabolites of *Colletotrichum* species. Mycopathologia, 150: 137-142.

Aref, H.L., B.F. Karima, J.P. Chaumont, A.F. Fekih, A. Mahjoub and K. Said, 2010. *In vitro* antimicrobial activity of four *Ficus carica* latex fractions against resistant human pathogens (antimicrobial activity of *Ficus carica* latex). Pak. J. Pharmaceutical Sci., 23: 53-58.

Arney, K.L., R. Tiernan and M.A. Judson, 1997. Primary pulmonary involvement of *Fusarium solani* in a lung transplant recipient. Chest., 112: 1128-1130.

Arora, D.R. and B. Arora, 2008. Textbook of Microbiology. 3rd Edn., CBS Publishers, New Delhi, India, ISBN-13: 9788123915494, Pages: 760.

Asadu, C.I.A. and F.O.R. Akamigbo, 1996. Performance of whole and cut setts of white yam (*Dioscorea rotundata*) for ware yam production in Southeastern Nigeria. Afr. J. Root Tuber Crop, 1: 1-43.

Ashraf, M.S. and T.A. Khan, 2007. Efficacy of *Gliocladium virens* and *Talaromyces flavus* with and without organic amendments against *Meloidogyne javanica* infecting eggplant. Asian J. Plant Pathol., 1: 18-21.

Awuah, R.T. and K.O. Akrasi, 2007. Suppressing of rot of yam caused by *Aspergillus niger* with a yam rhizobacterium. Afr. Crop Sci. Conf. Proc., 8: 875-879.

Banos, S.B., L.L.B. Necha, L.B. Luna and K.B. Torres, 2002. Antifungal activity of leaf and stem extracts from various plant species on the incidence of *Colletotrichum gloesporioides* of papaya and mango fruit after storage. Revista Mexicana de Fitopatologia, 20: 8-12.

Barcus, A.L., S.D. Burdette and T.E. Herchline, 2005. Intestinal invasion and disseminated disease associated with *Penicillium chrysogenum*. Ann. Clin. Microbiol. Antimicrob., 4: 21-21.

Boughalleb, N., M. Mhamdi, B. El-Assadi, Z. El-Bourgi, N. Tarchoun and M.S. Romdhani, 2010. Resistance evaluation of grafted watermelon (*Citrullus lanatus* L.) against fusarium wilt and fusarium crown and root rot*. Asian J. Plant Pathol., 4: 43-48.

Brenner, D.J., N.R. Krieg and J.T. Staley, 2005. Bergey's Manual of Systematic Bacteriology. 2nd Edn., Springer, New York, ISBN-13: 978-0-387-24145-6.

Cheesbrough, M., 2002. District Laboratory Practice in Tropical Countries. Cambridge University Press, Cambridge, UK., pp: 97-182.

Cowan, S.T. and K.J. Steel, 2002. Manual for the Identification of Medical Bacteria. 2nd Edn., Cambridge University Press, Cambridge, UK., pp: 51-120.

Ejechi, B.O. and D.E. Akpomedaye, 2005. Activity of essential oil and phenolic acid extracts of pepperfruit (*Dennetia tripetala* G. Barker) against some food-borne microorganisms. Afr. J. Biotechnol., 4: 258-261.

Ejechi, B.O., O.E. Nwafor and F.J. Okoko, 1999. Growth inhibition of tomato rot fungi by phenolic acids and essential oil extracts of pepperfruit (*Dennetia tripetala* G. Barker). Food Res. Int., 32: 395-399.

Ekefan, E.J., S.A. Simon, A.O. Nwankiti and J.C. Peter, 1999. Effect of intercropping on the incidence of *Foliar anthyacnose* and tuber yield of susceptible *Dioscorea alata* yam in Nigeria. J. Plant Prot. Trop., 12: 80-90.

Ezeibekwe, I.O., M.I. Opara and F.N. Mbagwu, 2009. Antifungal effect of aloe-vera gel on fungal organisms associated with yam (*Dioscorea otundata*, Poir) rot. J. Mol. Genet., 1: 11-17.

FAO, 1998. Food and Agriculture Organization Production Year Book. Food and Agricultural Organisation, Rome.

FAO, 2005. Food and Agricultural Organization. Statistics Division. Agricultural data of FAOSTAT. http://faostat.fao.org.

Gasink, L.B., P.H. Edelstein, E. Lautenbach, M.S. Ynnestvedt and N.O. Fishman, 2009. Risk factors and clinical impact of *Klebsiella pneumoniae* carbapenemase producing *K. pneumoniae*. Infect. Control Hosp. Epidemiol., 30: 1180-1185.

Gupta, V.K., A.K. Misra, R.K. Gaur, P.K. Jain, D. Gaur and S. Sharma, 2010. Current Status of *Fusarium* Wilt Disease of Guava (*Psidium guajava* L.) in India. Biotechnology, 9: 176-195.

Harborne, J.B., 1998. Phytochemical Methods. 3rd Edn., Chapman and Hall, London, ISBN: 0-412-57260-5, Pages: 302.

Junaid, S.A., A.O. Olabode, F.C. Onwuliri, A.E.J. Okwori and S.E. Agina, 2006. The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial wilt of tomato. Plant Pathol. J., 1: 71-73.

Kadowaki, T., H. Hamada, R. Ito, N. Hamaguchi, K. Sakai and J. Higaki, 2007. Chronic necrotizing pulmonary aspergillosis in the surgically treated lung. Nihon Kokyuki Gakkai Zasshi, 45: 372-376.

Khan, R., B. Islam, M. Akram, S. Shakil and A. Ahmad *et al*., 2009. Antimicrobial activity of five herbal extracts against Multi Drug Resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules, 14: 586-597.

Kierownik, 1990. Pulmonary aspergillosis caused by *Aspergillus niger*. Pneumonol. Pol., 58: 328-333.

Kubmarawa, D., G.A. Ajoku, N.M. Enwerem and D.A. Okorie, 2007. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr. J. Biotechnol., 6: 1690-1696.

Kubmarawa, D., M.E. Khan, A.M. Punah and M. Hassan, 2009. Phytochemical and antimicrobial screening of *Ficus platyphylla* against human/animal pathogens. Pac. J. Sci. Technol., 10: 382-385.

Kwon, J.H., S.S. Shen and H.J. Jee, 2008. Occurrence of blue mold on tomato caused by *Penicillium oxalicum* in Korea. Plant Pathol. J., 24: 87-89.

Kwon, J.H., S.W. Kang and C.S. Park, 2000. Occurrence of rhizopus soft rot on squash (*Cucurbita moschata*) caused by *Rhizopus stolonifer* in Korea. Plant Pathol. J., 16: 321-324.

Lehman, D., 2009. Triple sugar iron agar protocols. Proceedings of the American Society for Microbiology, October 5, 2009, USA.

Lopez, P., C. Sanchez, R. Batlle and C. Nerin, 2005. Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungi strains. J. Agric. Food Chem., 53: 6939-6946.

MacWilliams, M.P., 2009. Citrate test protocols. Microbe Library, American Society of Microbiology. http://www.microbelibrary.org/component/resource/laboratory-test/3203.

Mahgoub, E.S. and A.M. El-Hassan, 1972. Pulmonary aspergillosis caused by *Aspergillus flavusI*. Thorax, 27: 33-37.

Mehl, H.L. and L. Epstein, 2007. Identification of *Fusarium solani* f. sp. *cucurbitae* race 1 and race 2 with PCR and production of disease-free pumpkin seeds. Plant Dis., 91: 1288-1292.

Nahunnaro, H., 2008. Effects of different plant extracts in the control of yam rot induced by *Rhizopus stolonifer* on stored yam (*Dioscorea* sp.) in Yola, Adamawa State Nigeria. Agric. J., 3: 382-387.

Norman, M.J., A.T.C.E. Pearson and P.G.E. Searle, 1995. Tropical Food Crops in their Environment. Cambridge University Press, UK.

Ogaraku, A.O. and H.O. Usman, 2008. Storage rot of some yams (*Dioscorea* spp.) in Keffi and environs, Nasarawa State, Nigeria. Prod. Agric. Technol., 4: 22-27.

Okaka, J.C., P.A. Okorie and O.N. Ozon, 1991. Quality evaluation of sundried yam chips. Trop. Sci., 30: 265-275.

Okigbo, R.N. and I.A. Nmeka, 2005. Control of yam tuber rot with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale*. Afr. J. Biotechnol., 4: 804-807.

Okigbo, R.N. and M.I. Osuinde, 2003. Fungal leaf spot disease of mango (*Mangifera indica* L.) in Southeastern Nigeria and biological control with *Bacillus subtilis*. Plant Prot. Sci., 39: 70-77.

Okigbo, R.N. and U.O. Ogbonna, 2006. Antifungal effects of two tropical plant leaf extracts (*Occimum gratissimum* and *Afromonum meleguata* on Post harvest yam (*Dioscorea* sp.). Afr. J. Biotech., 5: 727-731.

Okigbo, R.N., 2004. A review of biological control methods of post harvest yam (*Dioscorea* sp.) in storage in south eastern Nigeria. KMITL Sci. J., 4: 207-215.

Okigbo, R.N., C.A. Agbata and C.E. Echezona, 2010. Effects of leaf extracts of *Aziradchita indica* and *Chromolaena odorata* on post harvest spoilage fungi of yams in storage. Curr. Res. J. Biol. Sci., 2: 9-12.

Okwute, S.K., 1992. Plant derived pesticidal and antimicrobial agents for use in agriculture. A review of Phytochemical and biological studies on some Nigeria plants. J. Agric. Sci. Technol., 2: 62-70.

Omoseyindemi, B., 2003. Plants as natural medicine. Proceedings of the 12th Annual Conference of the Botanical Society of Nigeria (BOSON) University of Lagos.

Parekh, J. and S. Chanda, 2007. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. Afr. J. Biomed. Res., 10: 175-181.

Priou, S., P. Aley, E. Chujoy, B. Lemaga and E.R. French, 1999. Integrated management of bacterial wilt of potato. CIP Slide Training Series (57 Slides and a 30 Pages-Guide in English and Spanish). International Potato Center (CIP), Lima, Peru. http://www.cipotato.org/potato/Pests_Disease/BacterialWilt/publications.htm

Sharma, V., S. Walia, J. Kumar, M.G. Nair and B.S. Parmar, 2003. An efficient method for the purification and characterization of nematicidal azadirachtins A, B and H, using MPLC and ESIMS. J. Agric. Food Chem., 51: 3966-3972.

Sonibare, M.A., A.A. Jayeola, A. Egunyomi and J. Murata, 2005. A survey of epidermal morphology in *Ficus* Linn. (Moraceae) of Nigeria. Bot. Bull. Acad. Sin., 46: 231-238.

Srivastava, S., V.P. Singh, R. Kumar, M. Srivastava, A. Sinha and S. Simon, 2011. *In vitro* evaluation of carbendazim 50% WP, antagonists and botanicals against *Fusarium oxysporum* f. sp. *psidii* associated with rhizosphere soil of Guava. Asian J. Plant Pathol., 5: 46-53.

Suleiman, M.N., S.A. Emua and A. Taiga, 2008. Effect of aqueous leaf extracts on a spot fungus (*Fusarium* sp.) isolated from compea. Am. Eurasian J. Sustainable Agric., 2: 261-263.

Teixeira, D.M., R.F. Patao, A.V. Coelho and C.T.D. Costa, 2006. Comparison between sample disruption methods and Solid-Liquid Extraction (SLE) to extract phenolic compounds from *Ficus carica* leaves. J. Chromatogr. A, 1103: 22-28.

Todar, K., 2011. *Pseudomonas aeruginosa*: The good, the bad and the deadly Todararica online textbook on bacteriology. Sci. Mag., Vol. 304.

Trease, G.E. and W.C. Evans, 2002. Pharmacology. 15th Edn., Saunders Publishers, London, pp: 42-44.

Ugwuanyi, J.O., 2008. Moisture sorption isotherm and xerophilic moulds associated with dried cocoyam chips in storage in Nigeria. Int. J. Food Sci. Technol., 43: 846-852.

Willey, J.M., L.M. Sherwood and C.J. Woolverton, 2008. Prescott, Harley and Kleins's Microbiology. 7th Edn., McGraw-Hill Higher Education, USA., Pages: 1088.

Yusuf, C. and B.A.O. Okusanya, 2008. Fungi associated with the storage rot of yam (*Discorea rotundata* Poir) in Yola, Adamawa State. J. Sustainable Dev. Agric. Environ., 3: 99-103.

Zerega, N.J.C., W.L. Clement, S.L. Datwley and G.D. Weiblen, 2005. Biogeography and divergence times in the mulberry family (Moraceae). Mol. Phylogenet. Evol., 37: 402-416.