Original Research Article

Antifungal activities of *Moringa oleifera* and *Ageratum conyzoides* against fungi causing deterioration of Plantain(*Musa paradisiaca* .L).

Abstract

Aim: This study investigated antifungal activities of ethanol and methanol leaves extracts of *Moringa oleifera* and *Ageratum conyzoides* on fungal pathogens identified in the deterioration of plantain.
Study design: This experiment was laid in a randomized complete block design. Rotten plantain Place and Duration of study: Department of Biology, The Polytechnic, Ibadan, Nigeria, between September 2022 and March 2023
Methodology: Samples were inoculated into Potato Dextrose Agar (PDA) for isolation of fungi causing spoilage. Fungal identification was based on cultural, microscopic and molecular techniques. The pathogenicity of the fungal isolates was proven by inoculating the isolates onto fresh, mature plantain fruits. The ethanol and methanol extracts of the selected botanicals were tested against the isolated fungi *in vitro*while the radial growth of the fungi were measured. Analysis of variance was carried out on the results obtained while means were compared using Least Significant Difference (P<0.05).
Results: The isolates obtained were identified as *Fusarium moniliforme* and *Rhizopus stolonifer*with the former being more virulent. In the *in vitro* screening, both botanicals showed a high degree of fungicidal activity by inhibiting the mycelia growth of the rot fungi.Ethanol extracts of both plants were more effective in the suppression of the fungal growth than the methanol extracts.
Conclusion: It is therefore concluded that the extracts of *Moringa oleifera* and *Ageratum conyzoides* inhibited the fungal pathogens causing spoilage of plantain *in vitro*.

Key words: *Moringa oleifera*, *Ageratum conyzoides*, Pathogenicity, in vitro

1. INTRODUCTION

Plantains (*Musa paradisiaca* L.) is one of the most important foodcropsin many African countries such as Nigeria, DRC,Cote d'Ivoire andNigeria (Adheka*et al*., 2018; Tenkuoano*et al*, 2019). It contributes to the fight against food security, alleviates hunger globally and serves as an important staple food for both rural and urban populace (USDA, 2012). It is used for making chips, flour, flakes, cakes, fried plantain, roasted plantain, and so many other forms of culinary provisions (Ojeniran*et al*., 2018). It is considered a highly nutritious fruit with the presence of a wide array of the essential nutrients and in the appropriate mix (Okareh*et al*., 2015; Oduje*et al*., 2015).

Despite the importance of the produce, itsproduction is threatened locally and globally by a complex of factors, namely: increase pest and disease pressures including postharvest rot amongst many others (Lescot and Ganry, 2010; Chabi *et al*., 2018; Tenkuoano*et al*., 2019). Consumption of fruit and vegetable products has dramatically increased in Nigeria by more than 40% during the past few decades. It is also estimated that about 30%of all fruits and vegetables produced is lost each year due to spoilage(Udoh *et al*., 2015; Adewuyi-Samuel *et al*., 2019b). In order to reduce postharvest loss, chemical control methods have been employed, but their use are restricted due to the hazardous effect caused to human health and the environment such as carcinogenicity, killing of non-target beneficial organisms, pollution and accumulation of toxic chemical residues (Ndakidemi*et al*., 2016).

These observed side effects of chemical treatmentnecessitates a need to employ methods that are safer and eco-friendly in controlling plant diseases both in the field and during storage. Theconcept of biological approach suchas the use of natural products (including plant extracts) inmanagement of disease in plant is considered asa good alternative to the use of syntheticfungicide due to their less or no negative impacts on man and the environment; as such, it is considered environmentally friendly, easy to use and affordable(Olowe *et al*., 2018; Oluwalana*etal*., 2022). This approach existed as folklore medicine among

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many indigenous populations and is gaining popularity as analternative means of controlling postharvest fungal rot of crops as reported in manyresearches (Odebode*et al*., 2006; Silva *et al*., 2014; Adewuyi-Samuel *et al*., 2019b; Oluwalana*etal*., 2022). Therefore, this research was aimed to examine the antifungalactivities of leaves of*Ageratumconyzoides* and *Moringaoleifera*on rotfungi of plantain.

2. MATERIALS AND METHODS

2.1 SampleCollection and Preparation

Fresh and healthy leaves of*Ageratumconyzoides* and *Moringaoleifera*were obtained from the Botanical Garden, University of Ibadan, Nigeria.The plant materials were authenticated at the Herbarium unit of Botany Department, University of Ibadan. The leaves were washed under running water toremove the adhering dust particles, they were air dried under shade for 14 daysandoven-dried to a constant weight and crispy texture. The dried leaves were ground to powder using a mortar andpestle and stored in sterile, air-tight, labeled containers until required (Okigbo and Omodamiro,2007). Samples of spoilt plantainfruits were purchased from Bodijaand Ojemarkets, Ibadan,Oyo State.

2.2 Isolation of fungi

Spoilt plantain fruits were washed under running tap and surface sterilized with 70% ethanol for30 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°C 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).

2.3 Molecular Characterization of Isolates

2.3.1 Fungal DNA Extraction

All isolates were grown in PDA for 7 days at 25 ºC. A small lump of mycelia was collected from the pure culture and transferred into a 1.5ml Eppendorf tube containing lysis buffer (400 mM Tris-HCL (pH 8), 60 mM EDTA- pH 8.0, 150 mM NaCl and 1% sodium dodecyl sulphate). The tube was briefly vortexed to disrupt the mycelia, after which it was maintained at room temperature for 10 min. Potassium acetate (150 µl) was added into the Eppendorf tube and this was followed by brief vortexing. The tube and contents were finally centrifuged at 13,000 × g for 1 min. The supernatant was transferred to another 1.5 ml Eppendorf tube and centrifuged again as described above after which it was transferred into a new 1.5 ml Eppendorf tube and an equal volume of isopropyl alcohol was added. The tube was thoroughly mixed by inversion and centrifuged at 13,000 × g for 2 minutes and the supernatant was discarded. The resultant DNA pellet was washed in 300 µl of 70% ethanol, after which it was spun at 10,000 rpm for 1 min and the supernatant was discarded. The DN2.3A pellet was air dried and dissolved in 50 µl of 1×Tris-EDTA. Purified DNA (1 µl) was used in 24 µl of PCR mixture (Liu *etal*. 2000).

2.3.2*PCR Amplification*

The extracted DNA was amplified by the Polymerase Chain Reaction. Each PCR mixture contained 10 µl of Red taq ready mix, 0.5 µl of each primer pair, 8 µl of analytical grade sterile water (Sigma-Aldrich) and 5 µl of genomic DNA in a total volume of 24 µl. The thermocycling program used was an initial denaturation (94 °C for 5 minutes), 30 cycles of denaturation (94 °C for 1 min), annealing (60 °C for 1 minute) and elongation (72 °C for 1 minute), then a stabilization (72 °C for 5 minutes) (Michaelsen *et al*., 2006). The genetic materials were electrophoresed on 2% agarose gel in Tris acetate- EDTA buffer and the gel was stained with ethidium bromide before observation in UV detector (Okoro *et al*., 2009).

2.3.3 DNA Sequencing

The sequence was determined at Laboratory of Mycology, Department of Environmental Science, University of Pavia, Pavia, Italy. The identity of the isolates were determined against known sequences in the GenBank using BLAST (Basic Local Alignment Search Tool).

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2.4 Pathogenicity Test

This was carried out using the method of Chukwura*et al*. (2010). Mature, fresh, healthy pineapple fruits were washed under tap water, rinsed with sterile distilled water, surface sterilized with 75% ethanol and blotted dry with filter paper. Holes were made in each of the fruits with a sterile 4mm Cork borer. Each of the isolated fungi was inoculated into the fruits and the cores of the fruits were aseptically replaced. The holes were sealed with petroleum jelly to prevent contamination. The fruits used as the control experiments were not inoculated. All experiments were carried out in triplicates and placed in clean polythene bags, moistened with wet balls of absorbent cotton wool to create a humid environment and incubated at room temperature for ten days. The samples were sectioned through the site of inoculation and examined for lesion development. Infected portions were aseptically transferred onto freshly prepared PDA to isolate microorganisms responsible for the infections.

2.5Solvent Extraction

Extraction of plant materials was carried out using modified procedures described by Okigboand Omodamiro (2007). About 400g of each plant powder were soaked in ethanol and methanol separately in the ratio 1:5 (w/v)for 3 days in tightly sealed vessels at room temperatureaccompanied by intermittent stirring using a sterile glass rod. The suspensions were thereafterfiltered through sterile muslin cloth, decanted and then filtered using sterile Whatman No. 1 filter paper inserted in a funnel. The filtrates were concentrated under vacuum to dryness using rotary evaporator at 40°C to obtain the crude extracts.

2.6In Vitro Antifungal Assay

Two millilitre (2ml) from each of the concentration of the extracts (500, 250 and 125 mg/mL) was dispensed into 9 cm diameter petri dish and agitated thoroughly with molten PDA forming Potato Dextrose Leaf extract Agar (PDLA). The agar extract mixture was allowed to solidify and then inoculated centrally with a 5mm diameter mycelia disc obtained from 5-day old culture of each of the test fungi. PDA plates inoculated with the test fungi but without the extract served as positive control while PDA plates inoculated with the test fungi with organic solvent served as negative control. All the plates were incubated at 28±2°C, after which the mycelia growth was measured for seven days at 24-hour interval. All the treatments were set up in triplicates (Oluwalana*et al*., 2022).

2.7 Statistical Analysis

Data collected were subjected to analysis of variance. Significant means were separated using Duncan Multiple Range Test at probability level of 0.05 (Cary, 2003).

3. RESULTS

Two isolates obtained from the sample were identified as *Fusarium moniliforme*and *Rhizopus stolonifer*.

3.1 Phylogenetic relationships between the isolated *Fusarium moniliforme* strain FM and otherstrains already documented in the NCBI

It was observed that this strain showed high similarities with six other fungi strains that were already documented on NCBI. This strain shares common ancestral lineage with other six fungi strains. It is more related to*Neocosmoporamacrospora*NR163291 and *Neocosmoporaperseae*NR164415. These two fungi share common ancestral lineage with *Neocosmoporagamsii*NR159548 which share common ancestral lineage with strain NR163531, NR130690, and NR164424 Figure.2. This was further affirmed in a molecular alignment study as presented in Figure 1.

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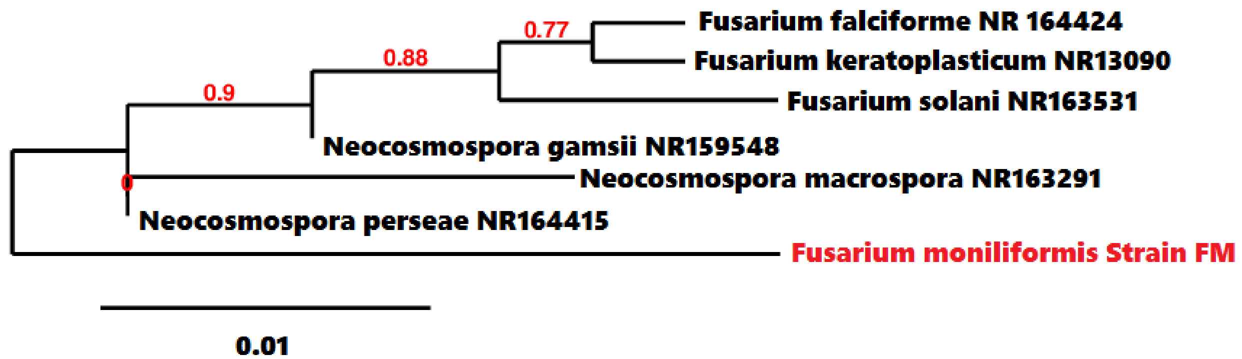

Figure 1: Phylogenetic relationship between the isolated *Fusarium moniliforme*strain FM and similar fungal strains already documented in NCBI

3.2 Phylogenetic relationships between the isolated *Rhizopus stolonifer* strain PF and other strains already documented in the NCBI

It was observed that this strain showed high similarities with six other fungi strains that were already documented on NCBI. This strain is closely related and shares common ancestral lineage with other fungus strain *Rhizopus koreanus*NR164543, they both shared common ancestral lineage with other strains *Rhizopus caespitosus*NR137056. This strain is similar to other groups of fungi; NR103636 and NR152951 which are also sharing common ancestral lineage to other fungal groups; NR103616 and NR103555 as shown in Figure 2. This was further affirmed in a molecular alignment study as presented in Figure 2

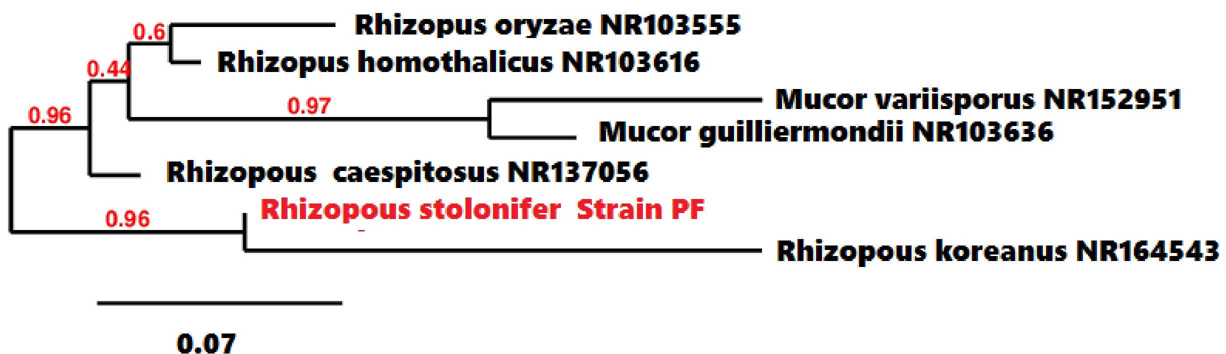

Figure 2: Phylogenetic relationship between the isolated *Rhizopus stolonifer*strain PF and similar fungal strains already documented in NCBI

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3.3 Pathogenicity of spoilage fungi causing rots in plantain

All the fungi isolated from naturally infected rotten plantain samples were able to re-produce similar rot symptoms when re-inoculated into healthy plantain fruits (Table 1). It was also observed from the table that both *F. moniliforme* and *R. stolonifer* caused varying levels of virulence. Visible rot symptoms were not observed on days 1-3 of incubation in the artificially infected plantain fruits as well as the control. Microbial deterioration of the plantain samples began on Day 4 with the sample inoculated with *R. stolonifer*showing a higher level of deterioration. As the incubation period progressed from Day 5 to the 7th day, the sample inoculated with *F. moniliforme*showed a greater deterioration and this was significant on the 7th day.

Table 1: Pathogenicity of fungal species isolated from rotten plantain samples

	Rot Formation (cm)						
Fungal Isolate	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Control	0.00	0.00	0.00	0.00[c]	0.00[c]	1.27[c]	1.97[c]
Fusarium moniliforme	0.00	0.00	0.00	1.00[b]	2.53[a]	2.90[ab]	3.17[a]
Rizhopusstolonifer	0.00	0.00	0.00	1.43[a]	2.10[ab]	2.53[b]	2.83[b]

3.4 Antifungal activity of ethanol and methanol extract of *Moringa oleifera* on isolated fungal

Table 2 shows the mycelia growth of *F. moniliforme* and *R. stolonifer* after treatment with ethanol and methanol extracts of *Moringa oleifera.* The ethanol and methanol extracts of *M. oleifera* were effective in the control of both *F. moniliforme* and *R. stolonifer* with improving efficiency as the concentration increased from 125mg/mL to 500mg/ mL. At low concentration (125mg/mL),the radial growth was high showing a poor inhibition of *F.moniliforme*and *R. stolonifer*in ethanol and methanol extracts of the botanical. Against both fungi, the ethanol extract was more effective than the methanol extract while*F. moniliforme* was more sensitive to *M. oleifera* extracts.

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Table 2: Antifungal activity of *Moringa oleifera* extract on isolated fungi

Fungal Isolate	Extracts	Concentration (mg/ml)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
			Radial Mycelial Growth Measurement (cm)						
Fusarium moniliforme	Ethanol Extract	125	0.43 ± 0.03^{abc}	0.65 ± 0.10^{bcd}	0.91 ± 0.34^{bcdef}	1.3 ± 0.28^{efg}	1.75 ± 0.27^{fg}	1.93 ± 0.74^{def}	2.37 ± 0.68^{ef}
		250	0.42 ± 0.03^{abc}	0.63 ± 0.08^{abcd}	0.84 ± 0.10^{bcde}	1.04 ± 0.14^{cde}	1.45 ± 0.15^{de}	1.63 ± 0.11^{cd}	1.82 ± 0.16^{cd}
		500	0.32 ± 0.03^{ab}	0.33 ± 0.04^{a}	0.35 ± 0.05^{a}	0.43 ± 0.10^{a}	0.53 ± 0.06^{a}	0.61 ± 0.07^{a}	0.77 ± 0.13^{a}
	Methanol Extract	125	0.42 ± 0.08^{abc}	0.53 ± 0.08^{abc}	0.88 ± 0.03^{bcdef}	1.22 ± 0.03^{defg}	1.65 ± 0.05^{ef}	1.85 ± 0.05^{de}	1.89 ± 0.07^{cd}
		250	0.3 ± 0.00^{a}	0.42 ± 0.10^{ab}	0.72 ± 0.03^{bcd}	0.98 ± 0.03^{bcd}	1.23 ± 0.15^{cd}	1.62 ± 0.03^{cd}	1.64 ± 0.04^{cd}
		500	0.33 ± 0.06^{abc}	0.53 ± 0.13^{abc}	0.7 ± 0.05^{bc}	0.73 ± 0.03^{b}	0.88 ± 0.08^{b}	1.05 ± 0.09^{b}	1.17 ± 0.15^{ab}
		Ethanol	0.37 ± 0.06^{abc}	0.64 ± 0.04^{bcd}	0.98 ± 0.03^{cdef}	1.4 ± 0.05^{fg}	1.65 ± 0.05^{ef}	2.27 ± 0.06^{efg}	2.82 ± 0.03^{fg}
		Methanol	0.37 ± 0.12^{abc}	0.7 ± 0.1^{bcde}	0.95 ± 0.05^{bcdef}	1.45 ± 0.13^{g}	1.99 ± 0.02^{gh}	2.55 ± 0.05^{gh}	2.55 ± 0.05^{efg}
		Control	0.42 ± 0.03^{abc}	0.88 ± 0.06^{de}	1.3 ± 0.1^{ghi}	1.83 ± 0.08^{h}	2.52 ± 0.13^{j}	2.94 ± 0.14^{h}	3.57 ± 0.10^{h}
Rhizopus stolonifer	Ethanol Extract	125	0.48 ± 0.16^{cd}	0.8 ± 0.00^{cde}	1.17 ± 0.15^{fgh}	1.78 ± 0.03^{h}	2.09 ± 0.11^{hi}	2.33 ± 0.21^{fg}	2.9 ± 0.1^{g}
		250	0.5 ± 0.05^{cd}	0.8 ± 0.05^{cde}	1.03 ± 0.06^{defg}	1.43 ± 0.03^{g}	1.75 ± 0.05^{fg}	1.97 ± 0.03^{def}	2.45 ± 0.05^{efg}
		500	0.42 ± 0.10^{abc}	0.52 ± 0.15^{abc}	0.62 ± 0.24^{ab}	0.82 ± 0.20^{bc}	1.1±0.1bc	1.27 ± 0.33^{bc}	1.6 ± 0.78^{bc}
	Methanol Extract	125	0.77 ± 0.03^{e}	0.98 ± 0.03^{ef}	1.43 ± 0.03^{hi}	1.90 ± 0.05^{h}	2.38 ± 0.03^{j}	2.68 ± 0.03^{gh}	2.79 ± 0.01^{fg}
		250	0.5 ± 0.05^{cd}	0.8 ± 0.05^{cde}	$1.1\pm0.1e^{fg}$	1.88 ± 0.03^{h}	1.98 ± 0.03^{gh}	2.37 ± 0.06^{fg}	2.4 ± 0.00^{efg}
		500	0.47 ± 0.08^{bcd}	0.75 ± 0.05^{cde}	0.87 ± 0.03^{bcdef}	1.15 ± 0.12^{def}	1.73 ± 0.04^{efg}	2 ± 0.00^{def}	2.1 ± 0.1^{de}
		Ethanol	0.6 ± 0.05^{d}	1.18 ± 0.18^{f}	1.48 ± 0.10^{i}	1.78 ± 0.03^{h}	2.3 ± 0.1^{ij}	2.65 ± 0.05^{gh}	2.88 ± 0.29^{g}
		Methanol	0.6 ± 0.05^{d}	0.9 ± 0.05^{de}	1.15 ± 0.13^{efgh}	1.78 ± 0.03^{h}	2.3 ± 0.1^{ij}	$2.57\pm0.8g^{h}$	2.9 ± 0.05^{g}
		Control	1.02 ± 0.18^{f}	1.65 ± 0.44^{g}	2.08 ± 0.38^{j}	2.58 ± 0.38^{i}	3.08 ± 0.038^{k}	3.53 ± 0.46^{i}	4.08 ± 0.8^{i}

Values are means of three replicates.Means having the same superscript letter are not significantly different ($\alpha_{0.05}$) according to Duncan MultipleRangeTest

3.5 Antifungal activity of ethanol and methanol extract of *Ageratum conyzoide* on isolated fungal

Table 3 shows the mycelia growth of *F. moniliforme* and Rhizopus stolonifer after treatment with ethanol and methanol extracts of *Ageratum conyzoides.* The ethanol and methanol extract of A. conyzoides were efficient as antifungal agents against both *F. moniliforme* and *R. stolonifer* compared with the control at various degrees of efficiency. The ethanol extract was efficient at both 250mg/ml and 500mg/ml concentrations against *F. moniliforme*. The most significant inhibition observed by the extent of radial growth in the Petri dish, occurred at the highest concentration and beginning from the third day of incubation. The methanol extract was also most effective at 500mg/ml concentration. The methanol and ethanol extracts showed similar inhibitions of *F. moniliforme* at 500mg/ml through the days of incubation. However, the inhibition at the 7th day were significantly different, with the ethanol extract being more effective. Against *R. stolonifer*, the ethanol extract of *A. conyzoides* was effective from the first 24 hours of incubation until the end of the incubation while the inhibition of the fungi by the methanol extract was most significant from the fourth day of incubation and both at the highest concentration. From the fourth day of incubation, the ethanol extract was more effective than the methanol extract as observed from the radial growth of the fungi.

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Table 3: Antigungal activity *Ageratum conyzoides*extract on isolated fungi

			Radial Mycelial Growth Measurement (cm)						
Fungal Isolate	**Extracts**	**Concentration (mg/ml)**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Fusarium moniliforme	Ethanolic Extract	125	0.3±0.00^{a}	0.58±0.19^{abcd}	0.93±0.32^{bcde}	1.35±0.41^{cd}	1.63±0.5.9^{d}	2.00±0.69^{de}	2.23±0.93^{defg}
		250	0.62±0.26^{def}	0.65±0.3^{abcd}	0.72±0.25^{abcd}	0.88±0.30^{ab}	1.08±0.24^{ab}	1.29±0.09^{bc}	1.78±0.29^{bcd}
		500	0.45±0.17^{abcdef}	0.48±0.19^{abc}	0.47±0.16^{a}	0.67±0.12^{a}	0.75±0.87^{a}	1.03±0.06^{ab}	1.52±0.23^{b}
	Methanolic Extract	125	0.32±0.03^{ab}	0.50±0.05^{abc}	0.62±0.04^{ab}	0.93±0.08^{ab}	1.25±0.05^{bc}	1.66±0.15^{cd}	2.1±0.1c^{def}
		250	0.35±0.05^{a}	0.5±0.09^{abc}	0.65±0.2^{abc}	0.93±0.08^{ab}	1.13±0.12^{b}	1.43±0.08^{bc}	1.65±0.05^{bc}
		500	0.33±0.06^{abc}	0.33±0.06^{a}	0.48±0.06^{a}	0.64±0.03^{a}	0.72±0.04^{a}	0.82±0.06^{a}	0.93±0.12^{a}
		Ethanol	0.37±0.06^{abc}	0.64±0.04^{abcd}	0.98±0.03^{cdef}	1.4±0.05^{cd}	1.65±0.05^{d}	2.27±0.06^{ef}	2.82±0.03^{h}
		Methanol	0.37±0.12^{abc}	0.7±0.1^{bcd}	0.95±0.05^{bcdef}	1.45±0.13^{de}	1.99±0.02^{de}	2.55±0.05^{fg}	2.55±0.05^{fgh}
		Control	0.42±0.03^{abcde}	0.88±0.06^{de}	1.3±0.1^{fg}	1.83±0.08^{f}	2.52±0.13^{f}	2.94±0.14^{g}	3.57±0.10^{i}
Rhizopus stolonifer	Ethanolic Extract	125	0.55±0.17^{cdef}	0.77±0.23^{bcd}	0.92±0.25^{bcde}	1.22±0.52^{bcd}	1.6±0.18^{cd}	2.03±0.17^{de}	2.48±0.23^{efgh}
		250	0.42±0.03^{abcdef}	0.67±0.08^{abcd}	0.75±0.00^{abcd}	1.07±0.12^{bc}	1.59±0.05^{cd}	1.9±0.05^{de}	2.3±0.22^{efg}
		500	0.38±0.02^{abc}	0.45±0.09^{ab}	0.68±0.14^{abcd}	0.95±0.22^{ab}	1.08±0.2^{ab}	1.25±0.13^{bc}	1.32±0.19^{ab}
	Methanolic Extract	125	0.63±0.08^{f}	0.83±0.03^{cd}	1.12±0.16^{ef}	1.5±0.00^{def}	1.88±0.12^{d}	2.1±0.1^{e}	2.73±0.03^{gh}
		250	0.52± 0.10^{bcdef}	0.72±0.03^{bcd}	1.02±0.08^{def}	1.45±0.05^{de}	1.78±0.03^{d}	2.07±0.06^{de}	2.23±0.15^{defg}
		500	0.4±0.09^{abc}	0.61±0.04^{abcd}	1.00±0.06^{cdef}	1.4±0.05^{cd}	1.7±0.09^{d}	1.84±0.10^{de}	2.03±0.06^{cde}
		Ethanol	0.6±0.05^{def}	1.18±0.18^{e}	1.48±0.10^{g}	1.78±0.03^{ef}	2.3±0.1^{ef}	2.65±0.05^{fg}	2.88±0.29^{h}
		Methanol	0.6±0.05^{def}	0.9±0.05^{de}	1.15±0.13^{ef}	1.78±0.03^{ef}	2.3±0.1^{ef}	2.57±0.8^{fg}	2.9±0.05^{h}
		Control	1.02±0.18^{g}	1.65±0.44^{f}	2.08±0.38^{h}	2.58±0.38^{g}	3.08±0.038^{g}	3.53±0.46^{h}	4.08±0.8^{j}

Values are means of three replicates. Means having the same superscript letter are not significantly different ($\alpha_{0.05}$) according to Duncan MultipleRangeTest

4. DISCUSSION

In this study, fungi found associated with the spoilage plantain fruits were identified as *Fusariummoniliforme* and *Rhizopus stolonifer*. Similar organisms have been isolated from watermelon, pepper, banana, plantain and pawpaw as reported by Adewuyi-Samuel *et al.* (2019), Akinyemi and Liamngee (2018), Udoh *et al.* (2015) and Singh *et al.*(2012). These pathogens may have infected the fruits through mechanical injury or wound during harvesting, transportation and storage thereby resulting in its reduction in quantity and quality.

All the isolated fungi were able to replicate the rot symptoms when re-inoculated on healthy plantain fruits, which proves that the isolates are responsible for the spoilage of the fruits. This was in line with the reports by Aslam*et al.* (2018) and Baiyewu*et al.* (2007) who stated that the above named organisms are actual pathogens of fruit crops. However, it was also observed that *F. moniliforme* had higher degree of deterioration than *R.stolonifer*. This agrees with the results obtained by Hassan *et al.* (2017) who reported *F. moniliforme* as one of the major causal agents of decay of banana fruit. Also, a similar observation was submitted by Baiyewu*et al.* (2007) who reported that *F. moniliforme* was among the major fungi responsible for rapid disintegration of pawpaw fruit.

The extracts of *Ageratum conyzoides* and *Moringa oleifera*had varying degree of effect on each of the fungal pathogen. The ethanol extracts were more effective in the inhibition of fungi growth in the *in vitro* experiments as compared to the methanol extracts of the tested botanicals. This is in agreement with the findings of Kaur *et al.* (2011) who reported that antifungal activity of the ethanol extracts appeared to be effective since ethanol could extract a wide range of bioactive components. Similarly, Adewuyi –Samuel et al. (2019a) affirms the efficiency of ethanol as an extraction solvent of phytochemical compounds. However, both extracts of ethanol and methanol were effective in controlling the growth of the spoilage pathogens in which there was reduction in mycelia diameter with increasing concentration of extracts. The work of Suraka *et al.* (2021) reported similar results where methanol and ethanol extracts of *Moringa oleifera* leaves were found to be effective against *Aspergillus flavus* and *Rhizopus stolonifer*. Similarly, Neela *et al.* (2014) reported the efficacy of ethanol leaf extract of *Moringa oleifera* against *Fusarium oxysporum* among tested plants. Rehman *et al.* (2020) submitted that the methanol extract of *Moringa oleifera* inhibited mycelia growth of the selected fungal species. The effect of these extracts on the tested fungal species lie in thebioactive constituents which they contain, including tannins, alkaloids, flavonoids phenols etc. (Anysor*et al.*, 2011; Onegbu*et al.*, 2020). In addition, Ageratum conyzoidesextract, significantly reduced the spoilage fungal biomass. Earlier studies have shown similar antifungal activities of ethanol leaf extracts of this weed against different fungal species such as *Fusarium lateritium*, *Fusarium solani*, *Cochliobolus lunatus* (Ilondu, 2013) and *Phytophthora megakarya* (Ndacnou*et al.*, 2020). Also, methanol extract of this botanical have been effective against different fungal pathogens such as *Fusarium oxysporum*and*Macrophominaphaseolina* as reported by Nur Hazirah*et al.* (2023) and Rashmi and Rajkumar (2011) respectively. According to Sukrasno (2017), the inhibition of the fungi might be due to the presence of antifungal compound in *Ageratum conyzoides* which have been reported to be coumarin. Coumarin have been widely distributed in several plant families such as Asteraceae in which Ageratum conyzoides belongs (Razavi, 2011).

5. CONCLUSION

This study revealed the potential of using biopesticides which are biodegradable and non-toxic as a substitute to the use of expensive synthetic chemicals and other methods for plant disease control. The use of botanicals as a means of bio control, reduces over-dependence on synthetic chemicals resulting in lower cost of production. The suppression of the fungal pathogens, strongly suggests that the active components of the indigenous plants are suiutablefor the control of fungi causing spoilage of plantain. Thus, it can serve as a better alternative to the use of synthetic fungicides. The botanicals used in this study are feasible and are not hazardous to the environment.

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