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The Effect of Melanthera Scandens and Mimosa Pudica on Fungi Causing Postharvest Deterioration of Cassava Root Tubers

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Abstract: Fresh cassava (*Manihot esculenta*) tubers unlike most other tubers of root crops are highly perishable. This study, therefore, investigated the antifungal activities of ethanol and hot water extract of *Melanthera scandens* (vine) and *Mimosa pudica* (touch me not) on rot fungi of cassava. Rotten cassava tuber samples were inoculated into Potato Dextrose Agar (PDA) for isolation of rot fungi causing postharvest deterioration of cassava tubers. The fungi obtained were identified using macroscopic and microscopic characteristics. The establishment of the pathogenicity of the fungal isolates was carried out by inoculating freshly harvested cassava tubers with the isolated fungi. The ethanol and hot water extract of the selected plants were tested against the isolated fungi both *in vitro and in vivo*. The isolates were identified as *Aspergillus niger, Aspergillus flavus, Penicillium notatum, Penicillium sp, and Fusarium verticillioides. A. flavus* being the most virulent with a percentage in severity of (27.15) on day 7 as compared with *Aspergillus niger, Fusarium verticillioides, Penicillium sp, and Penicillium notatum* which had (25.22, 17.17, 15.76 and 14.71) respectively. In the *in vitro* test the plants exhibited a high degree of fungicidal activity by inhibiting the mycelia growth of the rot fungi and *Mimosa pudica* was more effective in the suppression of fungal growth than *Melanthera scandens*. In the *in vivo* test there was a higher percentage reduction in diameter of rot when the plant extract was applied after twenty four hours of microbial inoculation directly to the cassava tuber. The extracts of *Melanthera scandens* and *Mimosa pudica* can therefore be used as a bio fungicide to extend the shelf-life of freshly harvested cassava tubers.

Keywords: Cassava, Fungicidal, Rot - Fungi, Postharvest Deterioration

1. Introduction

Cassava is the third largest source of food in the world, providing a basic diet for more than 800 million people [1]. It is a versatile crop and a raw material for many products such as starch, flour, tapioca, beverages, noodles, cassava chips and animal feed. It is also gaining prominence as an important crop for the emerging biofuel industry [2]. Nweke *et al.* reported that cassava play important roles in African development such as famine-reserve crop, rural staple food, cash crop for both rural and urban households also as raw material for feed and chemical industries [3]. The root is a physiological energy reserve with high carbohydrate content, which ranges from 32% to 35% on a fresh weight (FW) basis, and from 80% to 90% on a dry matter (DM) basis with small quantities of sucrose, glucose, fructose and maltose [4]. Nigeria is the highest producer of cassava globally, producing a third more than Brazil and almost double the production capacity of Thailand and Indonesia [5]. Nigeria in 2017 remained the highest producer of cassava in the world with about 59 million

tonnes [6]. However, Nigeria is not a strong participant in cassava trade in the international markets because cassava deteriorates fast once harvested, therefore it is targeted towards domestic food market.

Cassava can be stored underground (in-field storage) for a long period of time even after the stage of maturity. A negative effect occurring due to extensive in-field storage of cassava roots is their increased susceptibility to attack by pathogens as well as the reduction of extractable starch as cited by Onyewoke and Simoyan [7]. Once out of the ground, cassava has a shelf life of 24 to 48hrs after harvest [8]. Cassava undergoes postharvest physiological deterioration once the tubers are separated from the main plant. The tubers, when damaged, normally respond with a healing mechanism. However, the same mechanism, which involves the production of coumaric acids, initiates about 15 min after damage, and fails to switch off in harvested tubers [9]. It continues until the entire tuber is oxidized and blackened within two to three days after harvest, rendering it unpalatable and useless.

A major constraint faced by large-scale production and marketing of fresh cassava roots is the rapid postharvest deterioration (PPD) that occurs within two days after harvesting. This substantially reduces the eating quality, transportation range, and financial value of cassava [10-13]. Postharvest deterioration is one of the main obstacles currently preventing farmers from exporting cassava and generating income through foreign exchange. Postharvest root rot appears to be a combination of physiological and pathological factors. Deterioration of cassava root could be primary or secondary. Primary deterioration is a physiological change characterized by an internal root discolouration called vascular streak while secondary deterioration is that induced by microorganisms that cause rot. According to Arya, of all losses caused by plant disease, those that occur after harvest are the most costly [14]. Root rot is an economic important disease of cassava in Nigeria. Generally rot starts from the field and progress in storage. Fungi are the most important and prevalent microorganisms responsible for infecting and rotting a wide range of produce including fresh fruits, and causing economically important loses during transit and storage [15]. In order to meet the food demand challenges of our teeming economies, and to attain sufficiency and security in food production, food production must be matched adequately with their protection from spoilage and rots inducing organisms [16]. The exploitation of some plant extracts as alternative strategy in controlling postharvest fungal rot of crops has been emphasized by various researchers [17, 18, 16]. The use of natural products and organisms for the management of fungal diseases in plant is considered as a good alternative to synthetic fungicide, due to their less or no negative impact on the environment. Therefore, this research was aimed at examining the fungi- toxic activity of *Melanthera scandens* (vine leaf) and *Mimosa pudica* (touch-me-not leaf) on rot fungi of cassava.

2. Materials and Method

2.1. Collection of Plant Materials

Fresh and healthy leaf samples of *Melanthera scandens and Mimosa pudica* were collected from the Botanical Garden of the University of Ibadan, Nigeria. The plant materials were authenticated at the Herbarium Unit of Botany Department University of Ibadan. Diseased and healthy samples of cassava tubers were collected intermittently from cassava farms at Bakatari in Ibadan. Samples collected were aseptically packaged distinctly, well labelled and stored in a sterile collection kit. The packaged samples were transported to the Mycology/Pathology laboratory of the Department of Botany, University of Ibadan, for extraction of plant materials, isolation and characterization of fungi species associated with spoilage of harvested cassava tubers. After which further experiments were carried out.

2.2. Isolation and Identification of Cassava Rot Fungi

Target Pathogens were isolated from naturally infected root tubers, sectioned tissues from the cassava were taken from the healthy to diseased portions where the fungus are likely to be more active. The sectioned tissues were soaked in 0.1% sodium hypochlorite for 5min for surface sterilization and then rinsed in three changes of sterile distilled water. The infected tissues were aseptically transferred onto sterile filter paper using a sterile forceps and then blotted with filter paper for 3–5 minutes. After which, the infected tissues were aseptically inoculated onto freshly prepared plates of acidified potato dextrose agar (APDA). The inoculated media were incubated at 25±2°C in an incubator for 5-7 days and examined daily. The isolated fungi from infected tissues were purified by repeated culturing of the young active mycelia colony and incubated to obtain pure cultures of the pathogens. The fungi isolated from rotten cassava tubers were identified based on their colonial morphology and microscopic characteristics according to the method of Odebode *et al.* [19]. The cultures were maintained in PDA slants and stored at 4°C for further use.

2.3. Pathogenicity Test

The pathogenicity of the fungal isolates was established by using a modified method of Ubuala and Oti (20). Fifteen freshly harvested and healthy cassava roots were washed under running water and surface sterilized with 70% ethanol. Then it was divided into three equal groups. The first group were inoculated by using a 5mm diameter sterile cork borer, about 5cm long cylindrical core was removed from each root, disc of 7 day old pure culture of each isolates was picked from agar plates and placed in the bored holes on each tuber. The tubers were smeared with petroleum jelly (Vaseline) to completely seal the hole. The second group was inoculated with a 10^{-5} dilution of the isolated organisms at rate of 3mL of suspension per root. Equivalent volume of blank sterile distilled water was introduced into the third group following the same procedure. The three groups were incubated at room

temperature for 7 days. The cassava roots were examined on daily basis for the occurrence of rot. Re-isolation of the inoculated pathogens was done. All the treated tubers were put singly into sterile polythene bags and incubated at room temperature for 7 days. The cassava tubers was cut through and examined for rot at the end of the incubation period. An isolate was confirmed pathogenic, if it causes rot similar to that observed on the diseased cassava tuber from where it was isolated.

2.4. Extraction of Plant Materials

Fresh and healthy matured leaves of *Melanthera scandens*, and *Mimosa pudica* were thoroughly rinsed in running tap water before they were air-dried at room temperature for three weeks. The leaves were pulverised in a sterile electric blender and fine powder was stored in air-tight plastic container. Plant materials were extracted according to the method of Tijani *et al.* [21].

2.5. In Vitro Assessment

2.5.1. Effect of Plant Extract on Radial Growth of Fungal Pathogens

Two millilitre (2ml) from each of the concentration of the extract (100, 125, 150, 175, and 200 mg/ml) was dispensed into 9 cm diameter petri dish and agitated thoroughly with 13ml of melted 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 the active region colony of 5-day old culture of each of the test fungi using a sterile inoculating needle. PDA plates inoculated with the test fungi but without the extract served as the control. All the plants were incubated at 28±2°C, after which the mycelia growth was measured for seven days. All the treatments were replicated 3 times.

2.5.2. Mycelial Dry Weight Determination of Fungi Associated with Tuber Spoilage of Cassava Cultured on PDB Fortified with Plant Extract

The method employed was that of Odebode and Che [22], Potato Dextrose Broth (PDB) was used. A 10ml of sterilized acidified potato dextrose broth was dispensed into Mcartney bottles and 1 ml of each plant extract at different concentrations were aseptically pipetted into each bottles. A 5mm mycelia disk of 5 day old fungal isolate was inoculated into each of the bottles. The bottles were incubated at 28±2°C for 7days. The mycelia mats of the fungal isolate were harvested on the 7th day by filtering on pre-weighed Whatman No. 1 filter paper and oven dried at 80°C for 30 minutes. The weight of the mycelia was determined by subtracting the weight of the filter paper without the organism from the weight of the filter paper with mycelia. The treatments were replicated three times. Sterile distilled water and ethanol were used as control for the hot water and ethanol extracts respectively.

2.6. In Vivo Assessment of the Effect of Plant Extract on the Survival of Cassava Tuber Rot Fungal Pathogens

Fresh and healthy cassava tubers were washed with tap water, rinsed with distilled water and surface sterilized with 75% ethanol. Cylindrical disk of 6cm long and 5mm diameter were removed from each tuber with a sterile cork borer. Then a disc of five day old culture of the isolated fungus was transferred into the holes created in the cassava tubers. After 24hours, the inoculated tubers were treated with plant extracts at different concentrations and sealed with petroleum jelly. Tubers not treated with plant extracts but water and ethanol served as control for hot water and ethanol extracts respectively. The tubers were arranged in a Completely Randomised Design (CRD) with three replicates for each treatment.

2.7. Data Analysis

Data collected were subjected to analysis of variance using COSTAT 9.0 Statistical software and the homogeneity of means was determined using Least Significance Difference (LSD), Duncan Multiple Range Test (DMRT) and Tukey Kramer's Pairwise Comparison (TKPC) for highly sensitive means. The data were represented as means and standard deviation.

3. Results

The fungal pathogens isolated from rotten cassava tubers were identified as *Aspergillus niger, Aspergillus flavus, Penicillium notatum, Penicillium sp and Fusarium verticillioides.* All the fungi isolated reproduced rot symptoms when re-inoculated in fresh and healthy tubers of cassava (Figure 1) The pathogens induce different level of rot severity on the cassava tubers with *A. flavus* (27.15) being the most virulent while *P. notatum* (14.71) *was the least* amongst the isolates.

The radial mycelial growth measurement obtained from the treatment of fungal pathogens associated with root tuber deterioration of freshly harvested cassava tubers showed that both the ethanol and hot water extracts of *Melanthera scandens* and *Mimosa pudica* formulated at various concentrations (100mg/mL, 125mg/mL, 150mg/mL, 175mg/mL and 200mg/mL) were effective in the control of the pathogens.

Table 1 shows the mycelia growth of the rot pathogens on solid medium after treatment with ethanol and hot water extracts of *Melanthera scandens* and *Mimosa pudica* for 7days. In comparison with the control, there was significant difference between the ethanol extracts of *Melanthera scandens* and *Mimosa pudica* on *A. niger,* ethanol extract of *Mimosa pudica* was more effective than *Melanthera scandens* at concentrations of 100mg/mL, 125mg/mL, 150mg/mL, 175mg/mL and 200mg/mL. There was no significant difference between *Melanthera scandens* and *Mimosa pudica* at concentrations of 100mg/mL, 125mg/mL and 175mg/mL but there was significant difference at

concentrations of 150mg/mL and 200mg/mL. Also, there was no significant difference between the ethanol extract of the two plants at concentrations between 100- 150mg/mL on *A. flavus* until at concentration of 175mg/mL and 200mg/mL were signicant difference was recorded. No significant difference was also recorded for the hot water extracts of the botanicals for concentrations of 100,125 and 175mg/mL while at 150 and 200mg/mL there was significant difference on *A. flavus.* For the ethanol extracts of *M. scandens* and *M. pudica* significance difference was only recorded at 175 and 200mg/mL while for hot water extract signicance difference was recorded at 100 and 200mg/mL. At concentrations of 100 and 125mg/mL there was no significant difference between both ethanol extracts of the tested plants on *P. notatum* but signicant difference was recorded at concentrations of 150mg/mL, 175mg/mL and 200mg/mL, the hot water extracts only recorded signicant difference at 200mg/mL. Significant difference was also observed at concentrations of 100, 175 and 200mg/mL while there was no significant different at 125 and 150mg/mL between ethanol extracts of *M. scandens* and *M. pudica* on *F. verticilliodes,* there was significant difference at all the concentrations between the hot water extracts of the botanicals.

Table 2 reveals that the significant difference that abound in the level of performance of the aqueous hot water extracted bio-fungicides and the ethanol plant extract in the reduction of the pathogenic activities of *Aspergillus niger* associated with spoilage of harvested cassava tubers were largely negligible when compared vis-à-vis each other but highly significant when statistically compared to the performance of the control experimental setup at P≤0.05 for *Aspergillus niger* The result from the experiment further showed that the ethanol *Mimosa pudica* extract was more potent than *Melanthera. scandens* in the reduction of the dry mycelial mass of *Aspergillus niger* on treated PDB across all concentrations. There was no significant difference within the level of performance of the aqueous hot water extracted bio-fungicides and the ethanol plant extract in the reduction of the pathogenic activities of *Aspergillus flavus* when compared vis-à-vis each other but the level of significance was high when statistically compared to the performance of the control experimental setup for both water and ethanol at P≤0.05. The ability to induce weight loss on *Penicillium notatum* implicated in the postharvest deterioration of freshly harvested cassava tubers showed that there were significant differences in the level of performance of the aqueous hot water extracted bio-fungicides compared to the ethanol plant extract for all the botanicals employed for this experiment. Also, the level of reduction of the mycelial formation on PDA was greatly influenced, with a significant reduction in the growth capacity of *Penicillium notatum* associated with spoilage of harvested cassava tubers within the selected medicinal plant samples, and across the various levels of concentrations formulated for the bio-treatment analysis conducted on the pathogen and in direct comparison with the level of performance of the control experimental setup at P≤0.05 for *Penicillium notatum*. There was no disparity in the induced antifungal effect of the botanicals regardless of the method employed for the extraction of the active medicinal component of the plants. The dry mycelial weight assessment conducted showed that the mycelial mass of *Penicillium sp.* notable for spoilage of freshly harvested cassava root tubers was significantly reduced when statistically compared to the performance of the control experimental setup at P≤0.05 (Table 2). The result from the experiment further showed that it was only the performance of the aqueous hot water extract of *Mimosa pudica* at 100mg/mL (0.94±0.02cm) that was not statistically different P≤0.05 in the management of the *Penicillium sp.* when compared with all the controls setup for the experiment. The significant difference that abound in the level of performance of the aqueous hot water extracted bio-fungicides and the ethanol plant extract in the reduction of the pathogenic activities of *Fusarium verticilloides* associated with spoilage of harvested cassava tubers are largely negligible when compared vis-à-vis each other but highly significant when statistically compared to the performance of the control experimental setup at P≤0.05 for *Fusarium verticilloides*. The result gotten from the experiment further showed that it was only the extract of *Mimosa pudica* at 100mg/mL (0.87±0.01cm) obtained using hot water as the basic solvent that was not statistically different P≤0.05 in the management of the *Penicillium sp.* when compared with all the controls setup for the experiment.

Table 3 shows that the *in vivo* experiment conducted to determine the best medicinal plant extract suitable for the biocontrol of fungal pathogens responsible for the deterioration of freshly harvested cassava tubers showed that both the aqueous hot water extracted bio-fungicides and those extracted using ethanol as the basic solvent were very effective in the reduction of the disease symptoms induced by the pathological activities of these microbes at 200mg/ml which was the highest concentration in the *in vitro* experiments. It was observed that there was no significant difference in the performance of the ethanol extracts and that of the aqueous extracts in the control of *Aspergillus flavus*. However, there was a significant difference in the performance of the ethanol extracts from the aqueous hot water extracted botanicals in the control of *Aspergillus niger*, *Penicillium notatum*, *Penicillium sp.* and *Fusarium verticilloides* when compared vis-à-vis and the control experiment setup at P≤0.05.

The best recorded observations for the activity of the ethanol extract of the botanicals in suppressing the growth of *Aspergillus niger* was *Melanthera scandens* (1.92 ± 0.38^{e}) while the least was *Mimosa pudica* (0.60 ± 0.10^{f}) as compared to their individual control of (7.45 ± 0.00^{a}), (3.30 ± 0.00^{c}), respectively at day 7 when the experiment was terminated. The hot water extract also follow the same trend of *Melanthera scandens* (2.23 ± 0.25^{d}) and *Mimosa pudica* (0.73 ± 0.06^{f}). For *Aspergillus flavus* the most effective botanical was *Melanthera scandens* (0.50 ± 0.00^{c} and 0.80 ± 0.10^{c}), while the least was *Mimosa pudica* (3.77 ± 0.87^{b} and 3.83 ± 0.57^{b}) as compared to their

individual control of *Melanthera scandens* (6.05±0.00[a] and 6.20±0.00[a]), and *Mimosa pudica* (5.90±0.00[a] and 5.70±0.00[a]) with regards to ethanol and hot water extracts respectively. *Mimosa pudica* recorded the least activity among the two botanicals when tested on *Penicillium notatum*. The growth of *Penicilliunm sp.* was highly influenced by an inhibitory activity of the botanicals, and the medicinal plant with the highest inhibitory activity on this particular pathogen is *Mimosa pudica* (0.60±0.10[h]) and (0.83±0.06[g]), while *the least* was *Melanthera scandens* (0.50±0.00[i]) and (0.77±0.12[g]) as compared to their individual control of *Mimosa pudica* (3.90±0.00[b]) and (4.10±0.00[a]), *Melanthera scandens* (2.80±0.00[d]) and (3.00±0.00[c] for ethanol and hot water extracts respectively. The ethanol extract of *Mimosa pudica* had the lowest radial growth of (0.53±0.06[hi]) on *Fusarium verticillioides* while the least was *Melanthera scandens* (0.50±0.00[i]) as compared to their individual control of (4.30±0.00[a]), and (1.80±0.00[e]) respectively. However, the hot water extracts also follows the same trend, *Mimosa pudica* (0.70±0.00[f]), and (0.57±0.00[gh]) for *Melanthera scandens* as compared to their individual control of (4.30±0.00[a]), and (2.00±0.00[d]).

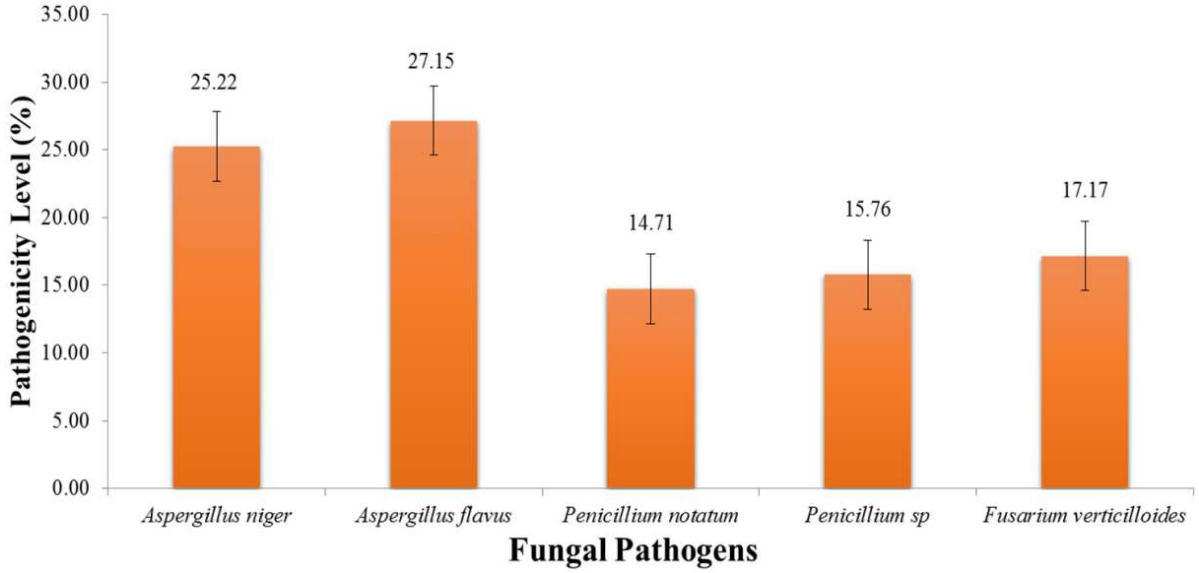

Figure 1. *The level of infection caused by the re-inoculated pathogens on healthy cassava tubers.*

Table 1. *Effect of The botanicals on fungal pathogen in solid medium (PDA) after 7 days.*

Pathogen	Solvent	Plant Extract	Radial Mycelial Growth Measurement (cm)					
			Treatment (mg/mL)					
			100	125	150	175	200	Control
Aspergillus niger	Ethanol	Melanthera scandens	1.30±0.02^{bc}	1.18±0.04^{bcd}	1.08±0.07^{cde}	0.91±0.01^{de}	0.44±0.01^{f}	4.33±0.21^{a}
		Mimosa pudica	0.82±0.03^{e}	0.75±0.01^{f}	0.58±0.05^{h}	0.54±0.03^{h}	0.39±0.03^{i}	4.40±0.05^{a}
	Hot Water	Melanthera scandens	1.43±0.10^{b}	1.38±0.06^{bc}	1.23±0.08^{bc}	1.12±0.03^{cde}	0.83±0.50^{e}	4.20±0.00^{a}
		Mimosa pudica	1.23±0.05^{b}	1.14±0.05^{c}	0.89±0.02^{d}	0.78±0.03^{ef}	0.66±0.03^{g}	4.39±0.03^{a}
Aspergillus flavus	Ethanol	Melanthera scandens	1.28±0.03^{cd}	1.24±0.04^{cd}	1.12±0.02^{de}	0.93±0.01^{ef}	0.83±0.03^{f}	4.24±0.04^{a}
		Mimosa pudica	0.72±0.01^{bc}	0.60±0.01^{d}	0.41±0.02^{ef}	0.23±0.03^{gh}	0.13±0.01^{h}	4.37±0.03^{a}
	Hot Water	Melanthera scandens	1.60±0.09^{b}	1.47±0.10^{bc}	1.27±0.08^{cd}	1.05±0.05^{def}	0.83±0.06^{f}	4.07±0.40^{a}
		Mimosa pudica	0.82±0.03^{b}	0.68±0.03^{cd}	0.46±0.03^{e}	0.31±0.01^{fg}	0.21±0.03^{gh}	4.29±0.22^{a}
Penicillium notatum	Ethanol	Melanthera scandens	1.19±0.06^{cd}	1.10±0.01^{cde}	0.68±0.03^{ef}	0.60±0.02^{f}	0.53±0.02^{f}	4.31±0.01^{a}
		Mimosa pudica	0.37±0.02^{de}	0.33±0.01^{def}	0.28±0.01^{efg}	0.21±0.01^{g}	0.11±0.01^{h}	3.98±0.03^{a}
	Hot Water	Melanthera scandens	1.82±0.64^{b}	1.48±0.28^{bc}	0.80±0.18^{def}	0.67±0.06^{ef}	0.50±0.26^{f}	4.18±0.33^{a}
		Mimosa pudica	0.51±0.02^{c}	0.41±0.02^{d}	0.32±0.01^{def}	0.26±0.02^{fg}	0.22±0.01^{g}	3.73±0.17^{b}
Penicillium sp	Ethanol	Melanthera scandens	1.29±0.05^{def}	1.23±0.07^{def}	0.98±0.02^{fg}	0.57±0.02^{h}	0.52±0.06^{h}	4.47±0.11^{a}
		Mimosa pudica	0.47±0.01^{d}	0.33±0.01^{e}	0.29±0.02^{e}	0.20±0.01^{f}	0.13±0.03^{g}	4.25±0.10^{a}
	Hot Water	Melanthera scandens	1.63±0.23^{c}	1.48±0.38^{cd}	1.39±0.29^{cde}	1.12±0.23^{efg}	0.90±0.10^{g}	3.57±0.12^{b}
		Mimosa pudica	0.56±0.02^{c}	0.52±0.01^{cd}	0.31±0.01^{e}	0.30±0.02^{e}	0.19±0.02^{f}	4.10±0.00^{a}
Fusarium verticilloides	Ethanol	Melanthera scandens	1.91±0.23^{b}	0.91±0.04^{cd}	0.31±0.03^{efg}	0.17±0.02^{fg}	0.07±0.12^{g}	3.52±0.11^{a}
		Mimosa pudica	0.35±0.01^{d}	0.35±0.01^{d}	0.22±0.01^{ef}	0.00±0.00^{h}	0.00±0.00^{h}	3.99±0.07^{b}
	Hot Water	Melanthera scandens	1.73±0.45^{b}	1.30±0.26^{c}	0.80±0.35^{d}	0.72±0.38^{de}	0.57±0.25^{def}	3.17±0.25^{a}
		Mimosa pudica	0.61±0.05^{c}	0.35±0.03^{d}	0.25±0.02^{e}	0.18±0.01^{fg}	0.14±0.01^{g}	4.38±0.08^{a}

Means with the same alphabets within each column not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT). Data collected were represented as "Means ± SD" only.

Table 2. *Effect of botanicals on fungal pathogen in liquid medium (PDB) after 7 days.*

Pathogen	Solvent	Plant Extract	Dry Mycellial Weight Measurement (cm) Treatment (mg/mL)					
			100	125	150	175	200	Control
Aspergillus niger	Ethanol	Melanthera scandens	0.84±0.01^{i}	0.82±0.01^{i}	0.81±0.01^{i}	0.80±0.01^{i}	0.79±0.01^{h}	1.08±0.00^{b}
		Mimosa pudica	0.91±0.01^{g}	0.83±0.01^{h}	0.82±0.01^{i}	0.81±0.00^{h}	0.79±0.01^{g}	1.00±0.00^{d}
	Hot Water	Melanthera scandens	0.86±0.01^{h}	0.85±0.01^{g}	0.84±0.00^{h}	0.83±0.01^{g}	0.82±0.01^{g}	1.12±0.00^{a}
		Mimosa pudica	0.93±0.02^{f}	0.91±0.01^{f}	0.86±0.01^{g}	0.83±0.02^{g}	0.82±0.01^{g}	0.96±0.00^{e}
Aspergillus flavus	Ethanol	Melanthera scandens	0.83±0.00^{f}	0.82±0.01^{gh}	0.81±0.01^{f}	0.80±0.01^{gh}	0.80±0.01^{g}	1.01±0.00^{c}
		Mimosa pudica	0.88±0.03^{e}	0.82±0.02^{gh}	0.81±0.00^{fg}	0.79±0.0i^{i}	0.79±0.00^{g}	0.99±0.00^{c}
	Hot Water	Melanthera scandens	0.83±0.01^{f}	0.83±0.00^{fg}	0.82±0.01^{f}	0.81±0.00^{fg}	0.80±0.01^{g}	1.05±0.00^{a}
		Mimosa pudica	0.88±0.03^{e}	0.82±0.02^{gh}	0.81±0.00^{fg}	0.79±0.01^{i}	0.79±0.00^{g}	0.99±0.00^{c}
Penicillium notatum	Ethanol	Melanthera scandens	0.81±0.01^{fg}	0.81±0.01^{j}	0.80±0.01^{i}	0.80±0.01^{i}	0.79±0.00^{i}	0.90±0.00^{e}
		Mimosa pudica	0.85±0.04^{efg}	0.81±0.00^{j}	0.80±0.00^{i}	0.80±0.00^{i}	0.79±0.00^{i}	0.92±0.00^{d}
	Hot Water	Melanthera scandens	0.82±0.01^{fg}	0.82±0.00^{i}	0.81±0.01^{h}	0.81±0.01^{h}	0.80±0.01^{h}	1.22±0.00^{a}
		Mimosa pudica	0.92±0.04^{bcd}	0.86±0.00^{g}	0.84±0.00^{g}	0.83±0.01^{g}	0.81±0.01^{g}	0.95±0.00^{c}
Penicillium sp	Ethanol	Melanthera scandens	0.82±0.00^{f}	0.80±0.01^{h}	0.80±0.00^{h}	0.80±0.01^{h}	0.80±0.01^{g}	0.99±0.04^{b}
		Mimosa pudica	0.91±0.04^{c}	0.85±0.01^{f}	0.84±0.01^{f}	0.82±0.01^{g}	0.79±0.01^{gh}	0.94±0.04^{d}
	Hot Water	Melanthera scandens	0.83±0.01^{f}	0.82±0.00^{g}	0.82±0.01^{g}	0.82±0.01^{g}	0.80±0.01^{g}	1.30±0.04^{a}
		Mimosa pudica	0.94±0.02^{cd}	0.91±0.02^{e}	0.85±0.02^{f}	0.84±0.02^{f}	0.81±0.02^{f}	0.99±0.04^{b}
Fusarium verticilloides	Ethanol	Melanthera scandens	0.83±0.00^{fg}	0.83±0.01^{e}	0.82±0.01^{e}	0.81±0.01^{e}	0.79±0.01^{fg}	0.88±0.00^{d}
		Mimosa pudica	0.86±0.05^{de}	0.82±0.01^{ef}	0.81±0.01^{f}	0.80±0.00^{e}	0.79±0.00^{g}	0.90±0.04^{c}
	Hot Water	Melanthera scandens	0.85±0.01^{ef}	0.84±0.01^{e}	0.83±0.00^{e}	0.82±0.01^{d}	0.82±0.01^{e}	0.95±0.04^{a}
		Mimosa pudica	0.87±0.01^{d}	0.86±0.01^{d}	0.85±0.01^{d}	0.83±0.01^{d}	0.80±0.01^{f}	0.91±0.04^{b}

Means with the same alphabets within each column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT). Data collected were represented as "Means ± SD" only.

Table 3. *The effect of botanicals on fungal induced cassava root tubers after 7days.*

Pathogen	Solvent	Plant Extract	Treatment	Control
Aspergillus niger	Ethanol	Melanthera Scandens	1.92±0.38^{e}	7.45±0.00^{a}
		Mimosa pudica	0.60±0.10^{f}	3.30±0.00^{c}
	Hot Water	Melanthera Scandens	2.23±0.25^{d}	7.50±0.00^{a}
		Mimosa pudica	0.73±0.06^{f}	3.40±0.00^{bc}
Aspergillus flavus	Ethanol	Melanthera Scandens	0.50±0.00^{e}	6.05±0.00^{a}
		Mimosa pudica	3.77±0.87^{b}	5.90±0.00^{a}
	Hot Water	Melanthera Scandens	0.80±0.10^{e}	6.20±0.00^{a}
		Mimosa pudica	3.83±0.57^{b}	5.70±0.00^{a}
Penicillium notatum	Ethanol	Melanthera Scandens	0.50±0.00^{e}	3.20±0.00^{a}
		Mimosa pudica	0.57±0.12^{de}	2.00±0.00^{c}
	Hot Water	Melanthera Scandens	0.57±0.06^{de}	3.20±0.00^{a}
		Mimosa pudica	0.57±0.12^{de}	2.30±0.00^{b}
Penicillium sp	Ethanol	Melanthera Scandens	0.50±0.00^{i}	2.80±0.00^{d}
		Mimosa pudica	0.60±0.10^{h}	3.90±0.00^{b}
	Hot Water	Melanthera Scandens	0.77±0.12^{g}	3.00±0.00^{c}
		Mimosa pudica	0.83±0.06^{g}	4.10±0.00^{a}
Fusarium verticilloides	Ethanol	Melanthera Scandens	0.50±0.00^{i}	1.80±0.00^{e}
		Mimosa pudica	0.53±0.06^{hi}	4.30±0.00^{a}
	Hot Water	Melanthera Scandens	0.57±0.06^{gh}	2.00±0.00^{d}
		Mimosa pudica	0.70±0.00^{f}	4.30±0.00^{a}

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

4. Discussion

It was observed from the research conducted on diseased cassava tubers that there was high occurrence of various species of *Aspergillus, Penicillium* and *Fusarium* on rotten cassava tubers. This suggests that these fungi might be responsible for the deterioration of cassava tuber after harvest. A similar observation was made by Okigbo *et al.* [23]. *Fusarium oxysporum*, *Fusarium solani*, *Aspergillus niger*, *Penicillium oxalicum* and *Botryodiplodia theobromae* were isolated from different rotten cassava tubers collected from rural farms across Nigeria. Onuegbu [24] also had a similar experience on dry rot of sweet potato were *Aspergillus niger* and *Aspergillus flavus* was isolated among other characterized fungi responsible for the decay of sweet potato.

All the isolated fungi were able to replicate the disease symptoms when inoculated on healthy cassava tubers, which proves that the isolates are responsible for the spoilage of the tubers. This was in line with the reports by Frank and Kingsley [25] who stated that the above named

organisms are actually pathogens of root and tuber crops. *Aspergillus flavus* had the highest percentage frequency of occurrence among all the pathogenic fungi followed closely by *Aspergillus niger* the least was *Penicillium notatum*. This finding was in accordance with the earlier report of Ubuala and Oti [20] who reported that *Aspergillus flavus* and *Fusarium solani* among other fungi were major causal organisms of cassava root rots. Also, a similar observation was submitted by Aidoo [26] who reported that most rots of yam tubers are caused by pathogenic fungi such as *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Botryodiplodia theobromae*, *Rhizoctonia* sp., *Penicillium oxalicum*, *Trichoderma viride* and *Rhizopus rodusus*.

Melanthera scandens and *Mimosa pudica* were tested on the isolated pathogens; the extracts had varying degree of effect on each of the fungal pathogen. The ethanol extracts were more effective in the inhibition of fungi growth in both the *in vitro* and *In vivo* experiments as compared to hot water extracts of the tested botanicals. This is in agreement with the findings of Kaur *et al.* [27] who reported that antifungal activity of the ethanol extracts appeared to be more effective than aqueous extracts since ethanol could extract a wide variety of active components as compared to aqueous.

However, all extracts (both water and ethanol) of the medicinal plant used were effective in controlling the growth of the cassava root rot pathogens. This is in agreement with the work of Okigbo and Nnemeka [28] where they reported the use of *Xylopia aethiopica* and *Zingiber officinale* in control of yam tuber rot caused by *Fusarium oxysporum*, *Aspergillus niger* and *Aspergilus flavus*. Tamilarasi and Ananthi [29] submitted that ethanol extract of *Mimosa pudica* exhibited antimicrobial activity against *Aspergillus flavus* and *Trycophyton ribrum* at all concentrations tested. The effectiveness of the extracts in retarding mycelia growth may be linked to the soluble nature of the phytochemicals present in the test plant materials as supported by Ubuala and Oti [20]. The ability of the plant extracts to be effective as an antimicrobial agent, might be due to the presence of phytochemicals which are secondary metabolites secreted by the plants.

The *In vitro* experiment showed that reduction in mycelia diameter increases with increasing concentration of extracts. Radial mycelia growth of fungi was lower in the ethanol extracts of all the botanicals than the hot water extract, this is supported by Odebode and Che [22] except for hot water extract of *Melanthera scandens* which was more effective in suppressing the growth of *P. notatum* than *M. scandens*. The effect of the plant extracts on mycelia dry weight followed the same trend as the radial mycelia growth effects. All the plant extracts had pronounced inhibitory effect on the mycelia growth and biomass in liquid and solid medium respectively. The inhibitory effect of *Melanthera scandens* and *Mimosa pudica* on the growth of *Aspergillus niger*, *Aspergillus flavus*, *Penicillium notatum. Penicillium sp.* and *Fusarium verticillioidies* was most effective at 200mg/ml.

The *in vivo* screening was carried out with ethanol and hot water extracts of *Melanthera scandens* and *Mimosa pudica* at 200mg/ml concentration which is the highest and most effective concentration when tested *in vitro* against the rot pathogens. Ethanol and hot water extracts t of *Melanthera scandens* to be more effective in the control of. *A. niger*, A. *flavus* and *P. notatum* than that of *Mimosa pudica*, this is in line with the report of Fagbohun *et al.* [30] who reported *Melanthera scandens* to be more effective as compared to *Occimum gratissimum* and *Leea guineensis* in the inhibition of the radial mycelia growth and reduction in dry mycelia weight of *Aspergillus flavus*. *Mimosa pudica* was the most effective in the control of, *Penicillium sp.* and *F. verticillioidies* of the tested botanicals. The variation in the results concerning the activity of the extracts may be due to the presence in the crude extracts of substances which exert antagonistic effects during the bio-assay procedure. The differences recorded in the fungitoxic activity of the extracts may also be attributed to the solubility of the active ingredients in water or the presence of inhibitors to the fungi-toxic principle.

5. Conclusion

Plant based extracts are biodegradable and are efficient in the reduction or eradication of fungal induced plant diseases. This study revealed the potential of using biological fungicides as a substitute to the use of expensive synthetic chemicals and other methods for plant disease control. The use of botanicals and microbes as a means of bio control, reduces over-reliance on synthetic chemicals resulting in lower cost of production. It is pertinent to engage disease control practices which are affordable by the majority of resource-poor farmers in our country. This will ensure substantial contribution of cassava to food supply and national economy. The contribution of the phytochemicals of the extracts in the control of the pathogens is indicated by both the *in vitro* inhibition of mycelia growth in both solid and liquid medium and also by the efficacy of the extracts against the colonies of the pathogens on the cassava roots. Ethanol and hot water extracts of the test plants can serve as an alternative to chemical control. The suppression of the fungal pathogens, strongly suggests that the active components of the botanicals and metabolites are suitable for postharvest control of cassava root rot. This study has revealed the potentials of botanicals tested in the control of fungi causing root rot of cassava tubers. The plants used in this study are readily available, easily accessible and are not hazardous to the environment. It is however recommended that further research should be directed towards discovering the mode of action of the specific active components of *Melanthera scandens* and *Mimosa pudica* responsible for the fungal inhibitory effect.

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