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BIOCONTROL POTENTIALS OF SELECTED PLANTS AGAINST SOME POST-HARVEST FUNGAL PATHOGENS OF YAM (*DIOSCOREA ROTUNDATA* P.) IN LAFIA, NASARAWA STATE, NIGERIA

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ABSTRACT

Ethanolic leaf extracts of Ficus sycomorus, Guiera senegalensis, Khaya senegalensis, Sclerocarya birrea, Azadirachta indica, Jatropha curcas and Tamarindus indica were evaluated for biocontrol potentials against selected post-harvest rot fungi of yam (Dioscorea rotundata) in Lafia, Nasarawa State, Nigeria. Rot fungi were isolated from decayed yam tissues by direct plating method. Pathogenicity test revealed that Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Fusarium sp., Rhizoctonia sp., and Mucor sp, induced rots in healthy yam tubers after 10 days of inoculation. Aspergillus niger was the most virulent, causing 18.7% tissue damage in infected yam tissues. All plant extracts showed effective inhibition of radial growths ranging from 89.51% to 93.77% on mycelia of Rhizoctonia sp. and Mucor sp., but all plant extracts showed moderate to effective inhibition ranging from 46.28% to 89.33% on mycelial growth of A. niger, A. flavus, A. fumigatus and Fusarium sp. The most significant fungitoxic effects of the extracts (P < 0.05) were observed with leaf extracts of F. sycomorus, G. senegalensis and A. indica at 10g/ml on all tested fungi. Phytochemical screening of ethanolic leaf extracts revealed the presence of saponins, tannins, flavaniods, alkaloids, and phenols in all evaluated plants except T. indica which showed the presence only of saponins and tannins. The biocontrol potentials of ethanolic leaf extracts of A. indica, F. sycomorus, G. senegalensis, J. curcus, K. senegalensis, S. birrea and T. indica, in the effective growth inhibition of the studied rot fungi is an affirmation of the possibility of incorporating these plant materials in the protection of mechanically injured yam tubers against rot fungi during storage.

Keywords: Biocontrol, Botanicals, Fungi, Post-harvest Rot, Yam

INTRODUCTION

Yam (*Dioscorea* spp.) belongs to the family *Dioscoreaceae* (IITA, 1993). These are perennial herbaceous vines cultivated for the consumption of their starchy tubers in West Africa, Asia, Latin-America, South East Asia, India, the Caribean and part of the Brazil. The most cultivated species in Nigeria are the *Dioscorea rotundata* (white yam), *Dioscorea cayanesis* (yellow yam) and *Dioscorea alata* (water yam) (Amusa, 1999). They are large plants; the vines can be as long 10-12m. The tubers often weigh about 2.5-5kg (6-12 pounds) (IITA, 1993).

Nutritionally, yams are mainly carbohydrate foods but contain about 1-2% dietary protein, which is high compared with other tropical root crops (Coursey, 1967). Yams are therefore able to provide a good proportion of protein requirement for man when consumed in large quantities (Coursey, 1967).

Out of the world production of over 30milloin tones per annum, Nigeria alone produces 22million tones (FAO, 1998). Despite this, the demand for yam tubers in Nigeria has always exceeded the supply. In Nigeria, it is eaten as boiled yam, yam pottage, fried yam, roasted yam, pounded yam and as Amala (Yoruba). It is also used as basic ingredient for snacks or made into floor for making into puree (Coursey, 1983; Okaka and Okechukwu, 1987).

Yam is prone to infections right from the seedling stage through harvesting and even after harvesting in storage. However, it has been estimated that an average of over 25% of the yield is lost annually to diseases and pests (Arene, 1987; Ezeh, 1998, FAO, 1998). Onayemi (1983) also reported that over 50% of the yam tubers produced and harvested in Nigeria are lost in storage. The disease causing agents reduce the quantity of yam produced and also reduce the quality by making them unappealing to the consumers. Different genera of fungi have been reported in association with storage deterioration of yam tubers. Post-harvest loss of root and tuber crops has been a very serious problem to farmers as more than 40% of their harvest may be lost due to decay (Olurinola *et al.*, 1992), and studies have shown that fungal rot is the greatest cause of roots and tubers loss in storage (IITA, 1993).

The principal fungal species associated with yam rot in Nigeria include *Botryodiplodia theobromate*, *Aspergillus tamari*, *Penicillim oxalicum*, *P. cyclopium*, *P. italicum*, *Fusarium oxysporium*, *F. solani*, *Rhizopus nigricans*, *Sclerotium rolfsii*, Muccor circinelloides, and Trichoderma viridae (Amusa and Baiyewu, 1999). In Nasarawa State, dry rot of yam caused by fungi is considered the most devastating of the entire storage diseases of yam (Ogaraku and Usman, 2008).

The use of chemical fungicides has helped in the control of postharvest rots, but the attendant problems of their nonenvironmental biodegradability, phytoxicity, pollution, development of resistance in target organisms and high cost (Okigbo and Odunikwe, 2009), have called for cheaper, sustainable and more environmentally friendly alternatives. Thus, this study explores the use of botanicals (plant extracts) in the control of fungal rot of yam tubers in Nasarawa State, Nigeria.

MATERIALS AND METHODS

Sources of Plant Material Rotted Yam Tubers

Yam (Dioscorea rotundata) tubers showing symptoms of soft and dry rots were obtained from four different yam storage facilities in Lafia, Nassarawa State. The diseased yam tubers were packaged in polyethylene bags and taken to the Postgraduate Laboratory of the Department of Plant Biology, Bayero University, Kano.

Botanicals

Disease-free leaves of 7 plants, namely; Azadirachta indica L., Sclerocarya birrea, Ficus sycomorus L., Guiera senesgalensis L., Jaftropha curcas L., Khaya senegalensis L., and Tamarindus indica used in this study were collected from wild and cultivated plants within Kano Metropolis, Kano State. Sampled plants were identified in the Herbarium Unit of Department of Plant Biology, Bayero University Kano, using taxonomic keys (Harrington, 1957).

Isolation and Identification of Fungal Pathogens

The isolation techniques were similar to those reported by Ritchie (1991) and Mahmoud and Al-Ani (2016). Small sections (2mm) of yam tissues containing the advancing margin of rot and adjoining healthy tissues were surface sterilized by immersion in 0.1% mercuric chloride solution for 1-2 minutes, and rinsed three times in sterile distilled water. The peeled and sliced periderm of the rotted yams were plated on Potato Dextrose Agar (PDA) and incubated at 27^oC for 7 days.

Fungal growths were sub-cultured on Potato Dextrose Agar (PDA) to obtain pure cultures. The resulting pure cultures were characterized and identified using cultural and microscopic examination of their growth morphology (Terna et al., 2019).

Pathogenicity Tests

Inoculation of Fungal Pathogens

Healthy yam tubes were washed with running tap water to remove soil and other debris. The tubers were surface sterilized with 1% sodium hypochlorite for 2 minutes, rinsed with sterile distilled water, and allowed to drain for 30 minutes. Both ends of surface sterilized yam tubers were each wounded once to a depth of 1cm using a sterilized 5mm diameter cork borer. Four millimeter discs of 7 days old pure cultures of fungal isolates

were used to plug the holes created in the tubers. Inoculated points were plugged with 7mm diameter yam plugs previously removed during wounding of yam tissues, and sealed air-tight with petroleum jelly. Surface sterilized yam tubers inoculated with 1ml of sterile distilled water served as control. Inoculated tubers were each enclosed in sterile polyethylene bags and incubated for 10 days at room temperature (25-37°C) in a microhumid environment provided by enclosing a sterile water soaked adsorbent cotton wool in each setup. The experiment was conducted in 3 replicates.

Assessment of Yam Tissue Rot

At the end of the 10 days incubation period, inoculated tubers were cut open along the points of inoculation using a sterile knife to obtain identical halves. Where positive, the length and girth of the rot area were measured in millimeters (mm) using a transparent meter rule (Okigbo and Ogbonna, 2006).

Preparation of Plant Materials for Extraction

Leaf samples were properly washed with tap water, rinsed with sterile distilled water and shade-dried. Dried leaf samples were separately pulverize in a laboratory mill, and sieved to obtain finer particles with higher surface area for extraction.

Extraction of Active Ingredients from Leaf Samples

The cold solvent extraction method reported by Harbone (1984) was used as follows; 25g, 50g, 75g and 100g portions of each powdered processed leaf samples were separately soaked in 100ml of absolute ethanol for 48 hours to yield 2.5g/ml, 5.0g/ml, 7.5g/ml and 10g/ml extract concentrations respectively. The extracts were filtered using Whatman No. 1 filter paper, and the filtrates recovered and stored in sterile conical flasks for biassay. In vitro Antifungal Assay of Plant Extracts

Effect of plant extracts on mycelia growth of test fungi was studied using the food poisoning technique reported by Salhi et al. (2017) with slight modifications. One milliliter of each plant extract concentration (2.5g/ml, 5.0g/ml, 7.5g/ml and 10g/ml) was dispensed per petri dish, and 9ml of sterile molten PDA added and gently mixed with plant extracts in each of the petri dishes, to give rise to PDA-extract mixture with corresponding 2.5g/ml 5.0g/ml 7.5g/ml and 10g/ml extract concentrations respectively. The PDA-extract mixtures were allowed to solidify and then separately inoculated at the center with a 4mm diameter mycelia disc obtained from the colony edge of 7 day old pure cultures of each of the tests fungi. The control experiment consisted of test fungi inoculated on sterile molten PDA mixed with sterile distilled water. All inoculated plates were incubated at 27°C, and the radial growth diameters of test fungi measured daily for 5 days, with a transparent meter rule. Colony diameter was taken as the mean growth along two directions on two perpendicular lines drawn on the reverse side of the plates. Inhibition of fungal growth by plant extracts was determined using a modification of the formula described by Himratul-Aznita et al. (2011) as follows:

Percentage Inhibition = $\frac{R1-R2}{R1} \times 100$

Where $R_1 = Radial$ growth diameter of pathogen in control

 $R_2 = Radial$ distance of pathogen in extract-incorporated agar plates.

Extracts were rated for their inhibitory effects using the scale described by Sangayomi (2004) as follows:

0%	= Not effective
> 0 - 20%	= Slightly effective
>20 - 50%	= Moderately effective
>50 - <100%	= Effective
100% inhibition	= Highly effective

Experimental Design

The experimental units were laid out in Randomized Complete Block Design (RCBD) comprising 6 fungal isolates, 7 plant extracts and four extract concentrations (6×7×4), administered in 3 replicates.

Data Analysis

The data collected were subjected to Analysis of Variance (ANOVA) and significantly different means were separated using Least Significant Difference (LSD) at 5% level of probability.

Phytochemical Screening of Leaf Extracts

The phytochemical components of leaf extracts were screened using the methods reported by Harbone (1984) and Trease and Evans (1989), as follows:

Test for Flavonoids:

Five milliliters of 10% dilute ammonia, followed by 1ml concentrated sulphuric acid were added to a portion of extract. The appearance of a yellow colouration that disappeared on standing indicated the presence of flavonoids.

Test for Saponins:

To 0.5g of extract was added 5ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil, shaken vigorously and observed for the formation of an emulsion.

Test for Tannins

About 0.5g of the extract was boiled in 10ml of distilled water in a test tube and filtered. A few drops of 0.1% ferric chloride were added and observed for brownish-green or a blue-black colouration.

Test for Alkaloids:

About 0.5g of extract was diluted in 10ml of 1% aqueous hydrochloric acid, boiled and filtered through a Whatman No. 1 filter paper. Two milliliters of dilute ammonia, followed by 5ml of chloroform were added to 5ml of the filtrate, and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10ml of acetic acid, followed by addition of 1ml of Draggendroff's reagent. The formation of a reddish brown precipitate was indicative of the presence of alkaloids.

Test for Phenolic Compounds

Small amounts of various extracts were taken separately in water and tested for the presence of phenolic compounds in 2ml dilute ferric chloride solution. The appearance of violet color indicated the presence of phenolic compounds.

Test for Phytosterols

One gram of each of the extracts were dissolved in 10 ml of chloroform and filtered. The filtrates were treated with few drops (3-4) of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of phytosterols.

RESULTS

The fungal pathogens isolated and identified from rotted yam tubers were Apsergillus niger, Aspergillus flavus, Aspergillus fumigatus, Fusanium sp, Rhizoctonia sp, Mucor sp, Geotrichum sp. and Cladosporium sp. (Table 1). The most frequently occurring fungi were Aspergillus niger, Fusarium sp., Mucor sp., and Aspergillus fumigatus, with 18%, 15%, 14% and 13% occurrences respectively.

Fungal isolates	Frequency of occurrence	Percentage occurrence
Aspergillus niger	20	18.7
Fusarium sp.	17	16.0
Mucor sp.	16	15.1
Aspergillus fumigatus	14	13.2
Aspergillus flavus	9	8.7
Rhizoctonia sp.	12	11.3
Cladosporium sp.	12	11.3
Geotrichum sp.	6	5.7

The result of pathogenicity test revealed that all the six test fungi (A. niger, A. fumigatus, A.flavus, Fusarium sp., Rhizoctonia sp., and Mucor sp.) were found to induce rot in healthy yam tubes after 10 days of inoculation (Table 2). The most virulent among the six fungi was Aspergillus niger causing 37.68% rot, followed by Aspergillus fumigatus (17.94%), Rhizoctonia sp. (13.65%), and Aspergillus flavus (12.94). The least virulent were Mucor sp. and Fusarium sp., inducing 8.96% and 8.86% rots respectively, in infected tissues.

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Fungi	Percentage rot
Rhizoctonia sp.	13.65
Aspergillus fumigatus	17.91
Aspergillus niger	37.68
Fusarium sp.	8.86
Mucor sp.	8.96
Aspergillus flavus	12.94

Table 2: Percentage Rot Caused by Test Fungi on	
Healthy Vam Tubers	

Radial growth of *A. flavus* was inhibited by all extracts of the evaluated plants (Table 3). Inhibition of fungal growth was concentration dependent, yielding the highest inhibitory activities at 10.0g/ml and the least at 2.50g/ml in all evaluated leaf extracts. Ethanolic leaf extracts of *Guiera senegalensis* gave the highest inhibition of the radial growth of *A. flavus* (62.72%). Differences in inhibitory activity of the evaluated plant extracts were significant ($P \le 0.05$).

Aspergillus flavus by Different Plant Extracts 7 Days after Inoculation							
		Concentration (g/ml)					
Plant Extract	2.50	5.00	7.50	10.00			
Ficus sycomorus	51.58	55.06	57.14	61.13			
Guiera senegalensis	47.88	55.54	59.53	62.72			
Jatrapha curcas	48.36	52.63	55.30	57.94			
Khaya senegalensis	51.07	54.26	55.86	56.34			
Azadirachta indica	47.32	52.11	55.30	59.53			
Sclerocarya birrea	46.28	53.71	56.10	60.09			
Tamarindus indica	46.76	52.11	55.86	58.49			
LSD	12.09	1.59	6.68	2.37			

 Table 3: Percentage Mean Radial Growth Inhibition of

 Aspergillus flavus by Different Plant Extracts 7 Days after Inoculatio

The inhibitory effect of ethanolic leaf extracts of the evaluated plants differed against *A. niger* (Table 4), and increased at higher extract concentrations. Ethanolic leaf extracts of *Ficus sycomorus, Guiera senegalensis* and *Sclerocarya birrea* at 10.0g/ml yielded the highest radial growth inhibition of *A. niger*. Differences in radial growth inhibition of *A. niger* by ethanolic leaf extracts of the evaluated plants were significant ($P \le 0.05$).

Aspergillus niger by Different Plant Extracts 7 Days after Inoculation						
		Concentration (g/ml)				
Plant Extract	2.50	5.00	7.50	10.00		
Ficus sycomorus	83.90	87.73	88.23	89.33		
Guiera senegalensis	85.89	87.31	87.98	89.33		
Jatrapha curcas	82.91	85.39	86.49	88.85		
Khaya senegalensis	80.59	86.14	87.56	89.02		

Table	e 4: Po	erce	ntage	e Mean	Radial	Growth	Inhibiti	on o	f

Science of ya birred 65.05 67.46 67. Tamarindus indica 78.28 85.99 86.	49 87.98
51.00 01.40 01.40 01.	
Sclerocarya birrea 83.65 87.48 87.	63 89.33
Azadirachta indica 84.57 87.31 87.	56 89.02

Results of percentage mean radial growth inhibitory effect of *Fusarium sp.* by ethanolicleaf extracts of different evaluated plants are presented in Table 5. *Ficus sycomorus* was the most fungitoxic, yielding the highest radial growth inhibition of *Fusarium sp.* (80.84%) at 10.0g/ml. Differences in radial growth inhibition of *Fusarium sp.* by ethanolic extracts of evaluated leaf samples were significant ($P \le 0.05$) at all other concentrations except at 2.50g/ml.

Table 5: Percentage Mean Radial Growth Inhibition of Fusarium sp.
by Different Plant Extracts 7 Days after Inoculation

Concentration (g/ml)					
Plant Extract	2.50	5.00	7.50	10.00	
Ficus sycomorus	71.99	74.02	76.85	80.84	
Guiera senegalensis	74.77	76.23	77.87	79.64	
Jatrapha curcas	72.56	75.66	76.10	77.30	
Khaya senegalensis	71.99	76.64	75.61	76.68	
Azadirachta indica	73.58	76.10	76.85	76.64	
Sclerocarya birrea	73.14	74.46	75.53	76.54	
Tamarindus indica	73.31	76.10	77.87	79.33	
LSD	16.84	1.11	1.10	2.00	

Radial growth inhibition of *A. funigatus* by ethanolic leaf extracts of different plants was concentration dependent (Table 6) yielding the least inhibitory activity at 2.50g/ml and highest at 10.0g/ml. Leaf extracts of *Guiera senegalensis* gave the highest inhibition of the radial growth of *A. funigatus* (83.39%) at 10.0g/ml concentration. Differences in fungitoxic activity of plant extracts against *A. funigatus* were significant at 5.0g/ml, 7.50g/ml and 10.0g/ml ($P \le 0.05$).

Table 6: Percentage Mean Radial Growth Inhibition of Aspergillus fumigatus by Different Plant Extracts 7 Days after Inoculation						
	Concentration (g/ml)					
Plant Extract	2.50	5.00	7.50	10.00		
Ficus sycomorus	78.69	80.11	81.24	82.17		
Guiera senegalensis	77.06	79.4	81.77	83.39		
Jatrapha curcas	76.94	79.09	80.13	80.72		
Khaya senegalensis	76.42	79.00	80.54	81.77		
Azadirachta indica	79.46	80.54	81.77	82.38		

Results of antifungal effects of ethanolic leaf extracts of different test plants against *Mucor sp.* (Table 7) showed effective biocontrol potential, attaining 90.00% inhibition in all evaluated plants. Leaf extracts of *Khaya senegalensis, Sclerocarya birrea,* and *Tamarindus indica* were the most effective, producing 93.77% inhibition of fungal radial growth at 10.0g/ml concentration of extracts. Differences in fungitoxic activity of assayed leaf extracts were significant at 7.50g/ml and 10.0g/ml concentrations (P \leq 0.05).

Table 7: Percentage Mean Radial Growth Inhibition of

Plant Extract	Concentration (g/ml)				
	2.50	5.00	7.50	10.00	
Ficus sycomorus	90.55	91.84	92.75	93.26	
Guiera senegalensis	91.74	92.29	93.12	93.39	
Jatrapha curcas	91.29	91.92	93.12	93.26	
Khaya senegalensis	92.39	92.75	93.12	93.77	
Azadirachta indica	91.79	92.25	93.12	93.67	
Sclerocarya birrea	92.39	92.84	93.12	93.77	
Tamarindus indica	91.74	92.36	93.22	93.77	
LSD	7.15	26.79	0.17	0.28	

Radial growth of *Rhizoctonia sp.* was inhibited the most at 10.0g/ml concentration of leaf extracts of all assayed plants (Table 8). Leaf extracts of *Azadirachta indica* and *Sclerocarya birrea* yielded the highest inhibition of the radial growths of *Rhizoctonia sp.* (93.35%) followed by *Ficus sycomorus* (93.16%). Variations in fungitoxic activity of different leaf extracts were significant at 10.g/ml concentration ($P \le 0.05$).

Table 8: Percentage Mean Radial Growth Inhibition of Rhizoctonia sp. by Different Plant Extracts 7 Days after Inoculation						
^	•					
Plant Extract	2.50	5.00	7.50	10.00		
Ficus sycomorus	90.90	91.68	92.78	93.16		
Guiera senegalensis	90.65	91.53	92.32	92.76		
Jatrapha curcas	89.51	91.09	91.20	91.87		

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Khaya senegalensis	91.14	91.53	92.32	92.72	
Azadirachta indica	90.74	91.53	92.02	93.35	
Sclerocarya birrea	89.85	90.29	92.22	93.35	
Tamarindus indica	90.06	90.74	91.68	92.32	
LSD	8.53	0.59	0.58	0.63	

Results of the qualitative phytochemical screening of Azadiraclita indica, Ficus sycomorus, Khaya senegalensis, Sclerocarya birrea, Jatropha curcas, Guiera senegalensis and Tamarindus indica (Table 9) revealed that all evaluated plants tested positive for the presence saponins and tannins. Tamarindus indica had the least phytochemical composition (Tannins and saponins), while Jatropha curcas had the highest composition of phytochemicals, testing positive for the presence of all assayed phytochemical constituents. Khaya senegalensis and Guiera senegalensis tested positive to all assayed phytochemicals except terpenoids and phytosterols. Flavonoids were absent in leaf extracts of T. indica, while alkaloids were absent in Ficus sycomorus and Tamarindus indica, while leaf extracts of A.indica, K. senegalensis, T. indica and G. senegalensis tested negative for the presence of terpenoids.

Phytochemical	Plant species						
	A.indica	F. sycomorus	K. senegalensis	J. curcas	S.birrea	T.indica	G. senegalensis
Flavonoids	+	+	+	+	+	-	+
Saponins	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+
Alkaloids	+	-	+	+	+	-	+
Phenols	+	+	+	+	+	-	+
Terpenoids	-	+	-	+	+	-	-
Phytosterols	+	-	-	+	-	-	-

 Table 9: Phytochemical Composition of Leaf Extracts of the Studied Plants

+ = Present

- = Absent

DISCUSSION

The fungal pathogens isolated from the rotted yam tubers in this study were similar to those reported by Ogaraku and Usman (2008) from rotted yam tubers in Keffi, Nasarrawa State. Several workers have also reported the isolation of these fungi from post-harvest rotted yam tubers (Amusa and Baiyewu, 1999; Eze and Madu, 1990). The isolation of more than one pathogenic organism from a particular yam tuber confirms the possibility of multiple infections whose effect may cause rapid rottening of root and tuber crops, as reported by Sangayomi (2004). In several instances, fungi gain entrance into yam tubers through natural openings and wounds created during harvesting, transportation, handling and marketing. However, Okigbo and Odunikwe (2009) noted that root and tuber crops at time of harvest may already be infested by pathogens derived from disease foliage, roots or mother tubers.

The present study showed the presence of fungitoxic compounds in *A.indica, F. sycomonis, G. senegalensis, J. curcus, K. senegalensis, S. birrea and T. indica*, since leaf extracts of these plants were able to inhibit the growth of the test fungi. Phytochemical screening of the plant extracts revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, and phytosterols. The medicinal and pharmacological potentials of these phytochemicals have been affirmed by the reports of several workers (Okigbo *et al.*, 2009; Okwu, 2004). The presence of bioactive substances also confer resistance to plants against bacteria, fungi and pests (Srinivasan *et al.*, 2001; Okigbo and Ajalie, 2005; Okwu and Joshia, 2006).

CONCLUSION

Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Fusarium sp., Rhizoctonia sp., and Mucor sp. accounted for significant post-harvest spoilage of yam tubers in the study area. The biocontrol potentials of ethanolic leaf extracts of A.indica, F. sycomonis, G. senegalensis, J. curcus, K. senegalensis, S. birrea and T. indica, in the effective growth inhibition of the studied rot fungi is an affirmation of the possibility of incorporating these plant materials in the protection of mechanically injured yam tubers against rot fungi during storage.

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