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Botanical alternatives in management of fungal pathogens of seedling blight of cashew (*Anacardium occidentale* L.)

Alaba Olaitan Adeji[1,*], Adefoyeke Olufunmilayo Aduramigba-Modupe[2]

[1]*Plant Pathology Section, Department of Crop Protection, Cocoa Research Institute of Nigeria, PMB 5244, Ibadan, Nigeria.*
[2]*Department of Crop Protection & Environmental Biology, University of Ibadan, Ibadan, Nigeria.*

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Abstract

Introduction: Cashew *(Anacardium occidentale L.)* is an important tree crop and seedling survival is pertinent to successful establishment. Cashew seedling is infected by blight pathogens causing more than 60% seedling lost, however pesticides residues related issues and high cost of chemical necessitate efficacy trials of aqueous extracts of *Mangifera indica*, *Azadirachta indica* and *Hyphtis suaveolens* evaluated *in-vitro* on associated pathogens.

Methods: Flora of blight-infected cashew seedlings was randomly collected from Cocoa Research Institute of Nigeria (CRIN) nursery between July and October, 2019. Mycoflora analysis was carried out in the plant pathology (Mycology) laboratory of CRIN. Antifungal assay of powdered *Mangifera indica*, *Azadirachta indica* and *Hyphtis suaveolens* were screened using aqueous extracts at 1:4 (w/v). Potato Dextrose Agar (PDA) amended with 1ml of 100%, 75%, 50%, 25%, and 0% of the extracts and Mancozeb (synthetic fungicide) as standard, 5mm mycelia mat disc of 10day old each of *Lasiodiplodia theobromae, Fusarium pallidoroseum and Macrophomina sp.* were placed at the centre of the amended media in triplicate and incubated 5-7days using complete randomized design (CRD). Mycelia extension inhibition and percentage growth inhibition (R) obtained.

Results: *Aspergillus niger, A. flavus, Fusarium oxysporium, F. pallidoroseum, Lasiodiplodia theobromae., Pythium sp., Rhizopus sp., Macrophomina sp.* and *Rhizotonia sp*. were isolated. *Fusarium pallidoroseum, L. theobromae* and *Macrophomina* sp. screened with the varied concentrations of botanicals showed reduction in mycelia diameter; *Mangifera indica* (31.50%), *A. indica* (48.70%) and H. *suaveolens* (25.86%) on *F. pallidoroseum* favorably competed with mancozeb (39%) at 25% concentration while only *M. indica* was significant on *L.theobromae(*64.12%)and *Macrophomina sp.*(40.29%) and significantly different from control (0%).

Conclusion: Aqueous extracts of *M. indica, A. indica and H. suaveolens* showed fungicidal potential on *F. pallidoroseum* and *M. indica was* significant on *L. theobromae* and *Macrophomina sp*.

Keywords: Cashew; Seedling Blight; Botanicals; Mycelia reduction

1. Introduction

Anacardium occidentale L. is an important tree crop, ranks third in international trade after hard nuts *(Cocos nucifera*) and almonds (*Prunus dulcis*) [1,2]. Cashew is indigenous to Brazil and commercially grown in Asia, Australia, South America and Africa [3]. This tree was introduced into Nigeria between 15 and 16 centuries by the Portuguese explorers purposely for erosion control and afforestation scheme of the defunct Eastern Nigeria [4]. Cashew is well adapted to seasonally wet and dry tropical climates and has the capacity to grow and yield satisfactorily on well-drained, light textured soils [5] with minimum inputs. Cashew is a prime tree crop of economic importance in Nigeria where more

*Corresponding author: Alaba Olaitan Adeji
Plant Pathology Section, Cocoa Research Institute of Nigeria, PMB 5244, Nigeria.

than 65% of the farming families who are small holder farmers depends on the crop as a major source of income [6, 7]. Nigeria became the largest producer of cashew in 2010 and the 6th largest producer of cashew with annual production of about 120,000 tonnes [8]. About 60-70% of the local production is commercialized of which about 90% is exported in the form of raw nuts [9]. The cashew industry provides up to 600,000 jobs which value at N24billion, as an export oriented-agricultural crop making an high contributor to Nigeria non-oil GDP [10]. Cashew is not only a fruit but food whose health must be aimed at protecting the crop from nursery to harvest.

Sustainable cashew production always starts by obtaining good planting materials from the nursery, in which nursery raised cashew seedling promote higher percentage of survival on the field than planting directly with seed [11]. Major cashew nursery diseases identified are seedling blight which consist of all pathogens associated with damping off, root rot, dieback and seedling wilt recording losses as high as 60-65% as of which 15-20% are caused by damping off pathogens alone [5]. Inflorescence blight and twig dieback caused by *Lasiodiplodia theobromae* been the main factor limiting cashew nuts production in Nigeria [12,13]. Nursery management of cashew seedlings includes the use of synthetic fungicides such as mancozeb and carbendazin [14].

Pesticide residues and heavy metal contaminations in agricultural products has necessitated researchers and chemical companies to develop safer and eco-friendly control measures for plant diseases and biologically active plant derived pesticides are expected to play an increasing significant role in crop protection strategies [14,15]. Botanical species have been known for their medicinal and antimicrobial properties, most of which have been used *in-vitro* and in-*vivo* in the control of various plant diseases and pests especially on cashew pathogen [13,15]. In Nigeria and many other developing countries, the use of many plant species both as pesticides and local medicines has been reported [13]. This study therefore, aims to isolate fungi associated with cashew seedling blight disease and evaluate antifungal characteristics of extracts of *Mangifera indica*, *Azadirachta indica* and *Hyphtis suaveolens* against these pathogens.

2. Material and Methods

2.1. Sample location and collection

Blight-infected cashew seedlings were obtained from cashew nursery sections of Cocoa Research Institute of Nigeria (CRIN), Ibadan. Wilted cashew seedlings were randomly collected between July and October 2019. Samples were collected aseptically in sterile Ziploc sample bags, made airtight and transferred to the mycology laboratory of Plant Pathology Section for microbial assay following standard techniques and procedures for isolation.

2.2. Fungi isolation

Collected seedling samples were sectioned into smaller pieces based on the plant parts; stem, root and leaf infected and showing typical lesions on the parts. The sectioned pieces of plant parts were surface sterilized using 10% sodium hypochlorite solution for 2 minutes, rinsed three times in sterile distilled water and blotted on sterile laboratory paper towel to remove secondary contaminants. Potato dextrose agar (PDA) was routinely prepared in the laboratory and sterilized plant parts were inoculated on acidified PDA for isolation. The inoculated plates were incubated at 28^{o}C+2^{o}C for 3-5days. Morphological and cultural appearance of colony observed and pure cultures were obtained and kept on PDA slant.

2.3. Pathogenicity Test

Fungal isolates were subjected to pathogenicity testing. Spore suspension of 14day old fungi isolates were harvested and prepare into suspension using sterile water. Spores were concentrated and 10ml of the spore suspension sprayed three weeks after sowing of cashew nuts in polythene bags under nursery environment. While control was sprayed with water and the seedlings were covered with transparent polythene bags for 7days. Seedlings were observed for seedling blight and sample were collected for re-isolation of the mycoflora after 6weeks of sowing as adapted from Nakpalo *et al.*, 2017 [16].

2.4. Preparation of Botanical extracts

The leaves of *Mangifera indica*, *Azadirachta indica* and *Hyphtis suaveolens* were dried and milled into powder; 100g leaf weighed, crushed and soaked in 400ml sterile water for 24hrs at 1:4 (w/v) according to Adeniyi and Olufolaji [13]. The soaked extracts of each botanical were filtered using a muslin cloth and the stock sterilized in water bath at 65^{o}C for 10 minutes.

2.5. Antifungal Assay

The *in-vitro* assay was carried out to determine the efficacy of extracts on radial mycelia growth of *Lasiodiplodia theobromae, F. pallidoroseum* and *Macrophomina* sp. The 10ml PDA were amended with 1ml of 100%, 75%, 50%, 25% and 0% concentrations of each extract of *M. indica*, *A. indica* and *H. suaveolens* replicated in triplicate. Five-millimeter mycelia disc from a 10day old culture of the pathogens was inoculated at the centre of the 85mm capacity Petri plates, replicated in triplicates, incubated at 28^{o}C$\pm$2^{o}C and arranged in complete randomized design (CRD).The medium with mycelia disc but with 0% extract served as control while medium with mancozeb (synthetic fungicide) serve as negative control. Records of mycelia extension of the pathogens were obtained from the 3rd day until control plates were covered. Mean mycelia inhibition and percentage mycelia reduction (R) were obtained according to [13]. Data were subjected to statistical analysis using SAS and mean were separated using Duncan multiple range test (DMRT).

3. Results and Discussion

The mycoflora associated with blight disease of cashew seedling isolated were nine fungi from eight genera. *Aspergillus niger, A. flavus, Fusarium pallidoroseum, F. oxysporium , Lasiodiplodia theobromae, Pythium* sp., *Rhizopus* sp., *Macrophomina* sp. and *Rhizotonia* sp. The fungi isolates occurred across all the infected cashew flora parts as shown in Figure 1. *Aspergillus niger* had highest occurrence at 27% follow by *F.pallidoroseum* at 18%, *Pythium sp.* and *Rhizopus* sp. have the least occurrence at 3% and 2% respectively.

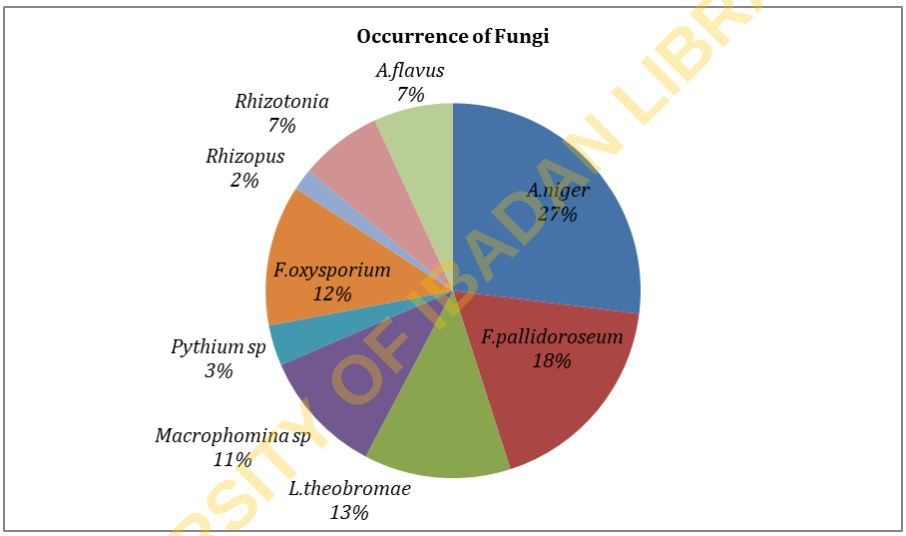

Figure 1 Occurrence of fungi isolates from cashew seedlings infect with blight

Pythium sp. isolated from the study is known to cause up to 5-25% loss in seedling while *Lasiodiplodia* sp., pathogen known to cause inflorescence and twig die back of cashew in Nigeria causes up to 60-65% yield loss [5, 13].

3.1. Pathogenicity Test

Lasiodiplodia theobromae, F. pallidoroseum and *Macrophomina* sp were re-isolated from cashew seedlings after the fungi isolates were subjected to pathogenicity test following Koch postulate.

3.2. Antifungal Assay

Mangifera indica, Azadirachta indica and *Hyphitis suaveolens* were screened for their antifungal potential against *L. theobromae, F. pallidoroseum* and *Macrophomina* sp. The aqueous extracts inhibited growth of *F. pallidoroseum* at all concentrations tested while only *M. indica* inhibits *L. theobromae* and *Macrophomina* sp. at 25%. Antifungal assay recorded highest mycelia reduction at 25% *M. indica* (31.50%), A. *indica* (48.70%) and *H. suaveolens* (25.86%) on *F. pallidoroseum* which was significant compared with the synthetic fungicide, mancozeb (39%). *Mangifera indica* on *Lasiodiplodia sp.* and *Macrophomina sp.* showed 64.12% and 40.59% mycelia reduction respectively, this is more significant when compare with mancozeb with 48.5% and 35.2% respectively and differ significantly from the control.

Azadirachta indica, M.indica and *H.suaveolens* significantly inhibited the mycelia of *F.palliodoroseum* while *M. indica* significantly inhibited *L.theobromae* and *Macrophomin*a sp only at 25% concentration compared with the control.

Azadirachta indica had 48.70% reduction in the mycelia extension of *F.pallidoroseum* at 25% phytoextract which exceed the synthetic fungicide, mancozeb at 39%. *Mangifera indica* had 64.12% reduction in mycelia extension on *L.theobromae* compared to the 48% of the mancozeb while *M.indica* had 40.59% reduction compared to 35% of mancozeb on *Macrophomina* sp.

The myeclial extension of *L.theobromae* and *Macrophomina* sp. were not significantly different from control when treated with *A. indica* and *H.suaveolens* at all tested concentrations similar to the report of Adeniyi and Olufolaji, **[13]** which reported that the mycelia growth of *L. theobromae* was not significantly inhibited when treated with *Azadirachta indica, Tridax procumbens, Vernonia amygdalina* and *Moringa oleifera* at all tested concentrations compared with the control.

Table 2 Mean Inhibition of Mycelia Extension and Percentage Reduction (R) of Fungi Mycelia by the Botanicals.

Extract conc. (%)				Mean Mycelial Diameter (mm)					
	Fusarium palliodoroseum			*Lasiodiplodia theobromae*			*Macrophomina sp.*		
	M. indica	*A. indica*	*H. suaveolens*	*M. indica*	*A.indica*	*H. suaveolens*	*M. indica*	*A.indica*	*H. suaveolens*
0	58.00bc	58.00bc	58.00bc	85.00a	85.00a	85.00a	84.50a	85.00a	85.00a
25	39.75c (31.50)R	29.75c (48.70)R	43.00bc (25.86)R	30.50c (64.12)R	85.00a (0)R	85.00a (0)R	50.50b (40.59)R	85.00a (0)R	85.00a (0)R
50	43.00bc (25.86)R	39.75c (31.47)R	51.25bc (11.64)R	84.50a (0.59)R	85.00a (0)R	85.00a (0)R	84.50a (0.59)R	85.00a (0)R	85.00a (0)R
75	44.00bc (24.14)R	41.75bc (28.02)R	51.00bc (12.07)R	84.50a (0.59)R	85.00a (0)R	85.00a (0)R	84.50a (0.59)R	85.00a (0)R	85.00a (0)R
100	43.25bc (25.43)R	43.50bc (25.00)R	47.25bc (23.71)R	84.50a (0.59)R	85.00a (0)R	85.00a (0)R	84.50a (0.59)R	85.00a (0)R	85.00a (0)R
Mancozeb	39c	39c	39c	48bc	48bc	48bc	35c	35c	35c

* Mean of three replication, ** Mean followed by the same letters in each column are not signoficantly different (P=0.05),Values in parenthesis represent percentage reduction (R) of Mycelial growth by the tested phytoextracts.

Mandal *et al*, [17] in their study on antimicrobial activity of leaf extract of *Hyphtis suaveolens* reported antimicrobial potential of its steamed extract on *Aspergillus* sp. and *Micrococus lutea*. And antimicrobial capacity of *Mangifera indica* was reported on aqueous and ethanolic extract on bacteria [18] which was similar to what was obtained in this study on the tested fungi. While the crude and alcoholic extract of *Azadirchta indica* reported to be fungicidal against *Rhizopus* sp. and *Aspergillus* sp. [19] was not significant on *L. theobromae* and *Macrophomina* sp. in this study. Aqueous extract of *Azadirachta indica* significantly inhibit mycelia of *Fusarium pallidoroseum*. Difference in percentage reduction of fungi mycelia at 25% of the phytoextracts were shown in figure 2.

Fusarium palliodoroseum was sensitive to all the phytoextracts and competed favourably with the synthetic fungicide. *Mangifera indica* significantly inhibit *Macrophomina* sp. and *Lasiodiplodia* sp. while *Hyphtis suaveolens* and *Azadirachta indica* were less. Antifungal potential of the phytoextracts varied with extracts concentrations which collaborate the report of Adeniyi and Olufolaji, [13] on their work on bio-efficacy of phytoextracts against *Lasiodiplodia theobromae.*

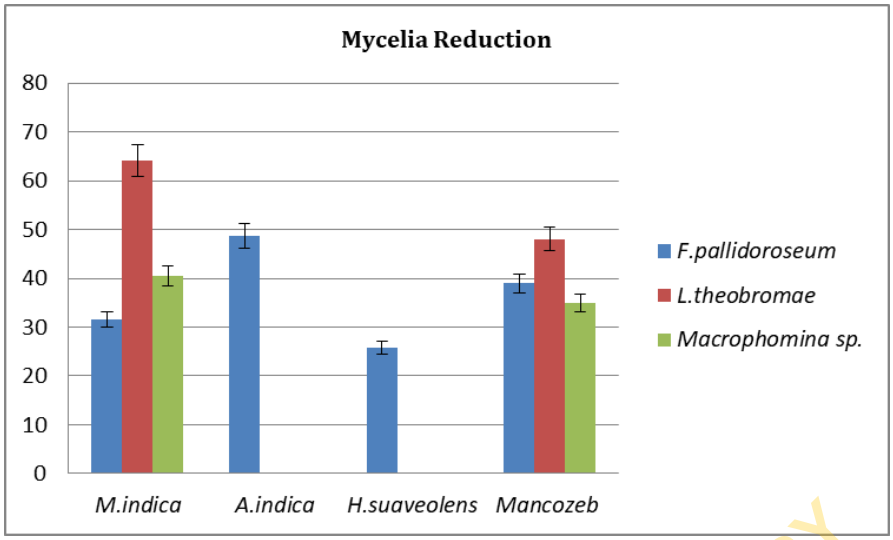

Figure 2 Percentage reductions in mycelia extension of Fungi pathogens by 25% concentration of the phytoextracts as compared to synthetic fungicide (Mancozeb).

4. Conclusion

Fungi associated with cashew seedling blight were isolated in the study**.** *Lasiodiplodia theobromae, Fusarium pallidoroseum and Macrophomina sp.,* the pathogens associated with cashew seedling blight was tested for their sensitivity to phytoextracts of *Mangifera indica, Azadirachta indica* and *Hypitis suaveolens*. Aqueous extracts of *M. indica, A. indica and H. suaveolens* exhibit antifungal efficacy on *F. pallidoroseum,* while only *M. indica* was effective against *L. theobromae* and *Macrophomina sp.* Fungicidal potential of the selected botanicals can be furthered proven *in-vivo*.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no known conflict of interest on the study.

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