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Evaluation of two plant leaf extracts on fungi associated with biodeterioration of cashew nuts in storage

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ABSTRACT

Three species of fungi isolated (Trichoderma viridae), Cephalosporium sp and Aspergillus nigar) were subjected to laboratory experiment involving in vitro control of the pathogens using plant extracts. Hot methanolic extractions from mango (Mangifera indica) and bitter – leaf (Vernonia amygdalina) leaves at 10%, 20%, 30% and 40% with the control (basal medium) concentrations tested on potato dextrose agar (PDA) for activity against mycelial growth were determined at 27±2^{0}C with three replicated plates. Vegetative growth values recorded were generally low compared with the control. Inhibitory action of the different extracts on vegetative growth increased with increase in concentration. Plant extracts readily available and affordable and environmentally friendly in the control of fungal disease. Plant extracts could serve as a reference material in further studies involving other pathogens of economic crops.

Key words: Plant extracts, fungi, cashew nuts, concentrations, inhibition.

INTRODUCTION

The cashew, *Anacardium occidentale* (L.), is a small to medium sized tree belonging to the family Anacardiaceae. The fruit is kidney –shaped achene with a hard grey-green pericarp. The seeds are the source of cashew nuts. World wide, cashew nuts are esteemed and highly priced food delicacy because of their pleasant taste and flavour. The post harvest processing, packaging and marketing have regulations adopted in major producing countries like India and Tanzania **[5]**.

Among the fungi predominantly encountered species in decreasing order of isolation from the non-disinfected nuts were *Aspergillus nigar, A. restrictus, A. Flavus, A. fumigatus, Rhizopus nigrians, R. arrhizus, Penicillium citrinum* and *P. digitatum* **[10]**.

Traditionally, man has used plants for health care in many countries. Many molecules of medicinal uses were originally derived from plants. However, in the 50(s), with the advance in anti-biotic and particularly the enormous development of synthetic organic chemistry, the use of herbs and herbal products created considerably scientific interests. Responses from traditional society indicate the species of bitter leaf have been very effective for treating of purgative skin disease and other related skin problems. The leaf extracts of this plant is used to combat fungal infections. Bitter leaf and mango leaf extracts in the family Asteraceae are widely grown and used in different parts of Nigeria popularly as food, ornamental and in traditional health care services **[9]** and **[6]**. The antifungal and antiviral property of bitter leaf was also reported by [**7**].

Consequence upon the above, and the need to source for alternative to chemical control, the role of higher plants as source of fungicides and their importance in controlling different plant pathogens are gaining prominence in view of the hazards and cost of agro-chemical plant extracts with their biodegradable and eco-friendly nature have shown some promise in recent years. The result could add to methods of control used by farmers, thereby reducing reliance on fungicides that are reported to predicate long term harmful consequences on environment, man and other wildlife.

MATERIALS AND METHODS

Leaves of Mango (*Mangifera indica*) and bitter – leaf (*Vernonia amygdalina*) are used. Fresh samples of each were used for the organic solvent (methanol) extractions following the methods of **[4]**. Each of the plant samples was washed thoroughly in cold running tap water, sun-dried for seven days. One thousand, five hundred grammes of each were homogenized using warring blender, and placed in 1000ml flasks containing 500ml methanol together with water in the ratio of 3:2. The mixtures were thoroughly mixed together and then placed in pots of water and heated to 100^0C for 30 minutes according to **[3]**. The filterates was concentrated using the vacuum evaporator so as to regenerate the methanol. It was filtered using Buckner funnel and dried solidified extracts weighed, which was 200g. The appropriate weights dissolved in 50ml distilled water to give the final concentrations of 10%, 20%, 30% and 40%, a modified method of **[4], [8]**. Streptomycin was added at the rate of $125mg^{-1}$ to each of the plant extracts to check bacterial contamination and kept for the *in vitro* assay.

The bioassay of the plant extracts was carried out by determining the effects of their concentrations on radial growth inhibition as described by Amadioha **[2]**. The PDA/crude extract medium was prepared by spreading 1ml of the extract separately on the surface of the solidified PDA in the Petri dishes. The control was PDA on which 1ml of sterile distilled water was spread on the surface. With the aid of sterile cork borer, 5mm diameter discs of seven-day old culture was cut from the pathogen grown on PDA, each placed, at the centre of the Petri dish containing the PDA/crude extract, and each treatment replicated three times. The whole setup was arranged in a completely randomized design. The incubation was carried out at 27 ± 2^0C and terminated at 6 days when the control mycelia had reached the edge of the Petri dish. Mean and percentage inhibition of mycelia growth determined.

RESULTS

The test fungi (*Trichoderma viridae*, *Cephalosporium* SP and *Aspergillus nigar*) were isolated from biodeteriorated cashew nuts in storage. The results showed that the two plant extracts had fungicidal properties, with bitter – leaf generally more effective in retarding vegetative growth than mango leaf extract. Even though visible mycelial growth was recorded in the concentration,

the extracts were less effective in controlling the mycelial growth in *Cephalosporium* sp, compared to what observed in *Trichoderma viridae* and *Aspergillus nigar* **(Tables 1 & 2).** These results showed that the inhibitory effects of the extract on mycelial growth increased with increase in contraction. The aqueous extract from bitter-leaf was inhibitory to mycelial growth in *Trichoderma viridae* durig the period of incubation with almost 100% inhibition. There was however, a significant difference between the control and other concentrations.

Table 1: Percentage of inhibition of bitter-leaf extract on the fungi (%)

Concentrations (%)	*Trichoderma viridae*		*Cephalosphorium sp*		*Aspergillus nigar*	
	Mean(cm)	%Inhibition	Mean(cm)	%Inhibition	Mean(cm)	%Inhibition
10	0.52	87.25	5.22	0.00	3.72	31.11
20	0.42	89.71	5.10	2.30	2.88	46.47
30	0.22	94.61	5.07	2.78	2.50	53.70
40	0.15	96.32	4.67	10.54	1.53	71.67
Control	4.08	-	5.22	-	5.40	-

Table 2: Percentage inhibition of mango leaf extract on the fungi (%)

Concentrations (%)	*Trichoderma viridae*		*Cephalosphorium sp*		*Aspergillus nigar*	
	Mean(cm)	%Inhibition	Mean(cm)	%Inhibition	Mean(cm)	%Inhibition
10	2.08	49.02	2.53	51.53	4.28	20.74
20	1.87	54.17	1.87	64.18	3.63	32.78
30	1.15	71.81	1.72	67.05	2.70	50.00
40	0.87	78.68	1.58	67.73	2.30	57.41
Control	4.08	-	5.22	-	5.40	-

DISCUSSION

Synthetic pesticides have been used in controlling fungal diseases and millions of naira is spent by tropical and sub-tropical countries in importing them. A greater number of farmers have no access to synthetic pesticides or cannot afford them. There is equally a reported complex of health and ecological problems caused by an improper use and over-use of pesticides. This has prompted the search for an alternative plant control. The two leaves extracts from each of the leaf plants used for the study – recorded retardation of vegetative growth of the fungi when compared with the pure culture. This may be as a result of the presence of active water solution antifungal principles associated with each of the plant leaves **[1]**. Also, this deduction could offer a partial reason for the popular use of bitter-leaf in traditional health care practices especially in all part of Nigeria for the treatment of various ailments **[6], [7]**. The mean radial growth values of the three fungi on media plates with different concentrations **(Tables 1 & 2)** supports the percentage inhibition noticed, which is a reflection in the water soluble antifungal element in their respective leaves. From the result, it was observed that the aqueous extracts used for the study recorded retarded or inhibited of mycelial growth of the fungi *in vitro*. The water – soluble antifungal principles in the plants are responsible for the antifungal activities. The facts that these plants used in this study are easily available, with easy method of extraction, they can be exploited in the control of cashew nut biodeterioration disease.

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