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ANTIFUNGAL ACTIVITY OF THE PLANT *COSTUS AFER* EXTRACT ON YAM (DIOSCOREA SPECIES) ROT PATHOGEN IN OWERRI. SOUTH-EAST NIGERIA

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ABSTRACT

The study was carried out to determine the effect of costus afer on pathogens causing yam rot in Owerri. Infected yams were sampled from two markets within Owerri. Two fungi pathogen isolated and identified were: Aspergillus niger and Aspergillus flavus. Three different concentrations of costus afer were obtained. 10, 20 and 30%phytochemical constituents of costus afer extracts was also evaluate. It contains phenols, flavonoids, quinones, alkaloids and tainnins. Extracts of costus afer was efficient in inhibiting the growth of Aspergillus niger and Aspergillus flavus. The extracts showed significant difference at 5% probability level.The highest antifungal activity was observed with 30% costus afer extract and ethanol had the highest inhibition 89% and 90%, when compared to aquae's 81 and 82% and crude 85 and 87% respectively.

The result of this study shows the possible use of plant extracts in the manangement and control of yam rot.

KEYWORDS: Costus afer, Pathogens, Extracts, Aspergillus sp, Phytochemical

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INTRODUCTION

Yam belonging to the family Dioscoreaceae and a monocotyledonous plant. It is a herbaceous annual climbing plant with edible underground tubers (orkwor et. al., 1998). There are hundreds of wild and domesticated Dioscorea species. The white Guinea yam. (*D.rotundata*) is the most important species especially in the dominant yam production zone in West and central Africa. The yellow yam (*D.cayenensis*) and water yam (*D. alata*) is the second most cultivated species originated from Asia and is the most widely distributed species in the world. (IITA, 2009). Nigeria alone produced 37.0 million tones in the year 2007 which makes her to be the highest producer.(llTA, 2009).

Yam post harvest loss has been a serious problem to farmers as a result of decay on the tuber (Olurinola et. al., 1992). It is the least perishable among the common root and tube crops and some measures have been employed to protect it from losses caused by pathogenic agent (Bediako et. al., 2007). Due to the problem at post harvest on yam, some botanicals have been exploiting to serve as fungicide and antibiotics that are environmental friendly and safe (Okigbo et.al., 2006). Previous report has indicated that *costus afer* extracts contains some bioactive ingredient that has inhibitory effects on some microorganisms (Anyasor et.al., 2010). *Costus afer* is commonly called bush cane. The stem is crew to reliance cough and sores (Edeoga et. al., 2000). The treatment and control of tube yam rot is due to the high cost of effective chemicals. The need to protect yam against diseases helps to enhance the production of the crop in the country. The present study aims at determining the effect of costus after extract on growth of some rot pathogens of yam.

MATERIALS AND METHODS

Fresh stem of costus afer was collected from the forest along Port Harcourt Road for the study and identified by Prof. S.E. Okeke, a plant taxonomist in the department of Plant Science. and biotechnology, Imo State University, Imo State. Yam tuber (Rot) used for this study was purchased from Relief market, Owerri, Imo State.

Media Preparation

Potato Dextrose Agar, was used and prepared according to the manufacturer's instructions and was dispensed into sterile Petri dishes for culture. The rotted yam was scraped using a sterilized knife at the infected areas, after which each small piece was placed on the PDA. The plates were then sealed with paraffin to prevent contamination, and then incubated with replicates. Examined for growth, sub-cultured and pure cultures were used for identification. The isolates were observed and laboratory manual by (Barnett, 1998).

Extraction of Plant (*Costus Afer*)

It was carried out according to the method described by (Harbone, 1973). The bark and leaves were removed from the plant stem. The stem was cut into tiny pieces. The difference mixture was filtered through Whatman filter paper No. 1. Then, the filtrate was concentrated under reduced pressure at 80^0C using rotary evaporator and stored in the refrigerator at 4^0C ready for use.

Three different aqueous, crude and ethanol extract concentrations were prepared by blending 10g, 20g and 30g of plant stem in 100g each of distilled water and 100ml of 10%, 20% and 30% respectively. The raw material was blended without any liquid.

Effect of *Costus Afer* Extract

The effect was determined in –vitro on the isolate identified. 9ml of PDA media was dispensed into stem, the Petri – dishes and allowed to solidify. Two diagonal lines were made at the bottom of each plate to determine the center of the plates. One milliliter of each plant extract at different concentration (10%, 20%, and 30%) was dispensed into the Petri-dish with PDA and then inoculated with a 5mm diameter mycelia disc obtained from a 4 day old culture of each test fungi. The control consists of no extracts with each test fungi. All the treatments and the control were replicated thrice and were inoculated at 27^0C for five (5) days.

The radial growths of the pathogens were measured in each case by using a ruler in cm to measure the zone of inhibition of the organisms. Then, the percentage of inhibition of growth of fungus was calculated as follows according to (Odebode et. al., 2004),

$$\% \text{ of inhibition} = \frac{F1 - F2 \times 100}{F1} \quad \frac{}{1}$$

Where FP = % inhibition of fungal

F1 = % inhibition of control growth

F2 = % inhibition of growth treatment.

Phytochemical Screening

The phytochemical screening for Phenols, Alkaloid, Saponins, Flavonoid and was determined by using the methods described by Trease and Evans (1989).

Data Analysis

The data collected from the study were subjected to analysis of variance (ANOVA) using fisher's least significance difference (LSD) at 0.05 level of probability.

RESULTS

Two differences of fungal species were isolated from the yam rotted samples. The fungi that were isolated are *Aspergillus niger* and *Aspergillus flavus*. The two isolated pathogenic fungi were tested in vitro against *costus afer* extract at different concentrations on mycelial growth of *Aspergillus flavus* and *A. niger*.

Table 1: Effect of Different Concentrations of Extracts on the Growth of *Aspergillus. flavus*

Extracts	10%	20%	30%
Aqueous	9.26	45.54	84.26
Ethanol	20.37	52.68	90.81
Crude	16.67	50.00	87.04
Control	00.00	00.00	00.00

Table 2: Effect of Different Concentrations of Extracts on the Growth of *Aspergillus niger*

Extracts	10%	20%	30%
Aqueous	11.61	69.30	82.46
Ethanol	19.54	72.05	89.47
Crude	15.18	64.91	85.96
Control	00.00	00.00	00.00

On the inhibitory effects of the costus afer extracts concentration on mycelia growth of the pathogens, there were significant at differences at (P = 0.05).

At 10% extract concentration *Aspergillus niger*, the Aqueous, Ethanol and crude extracts of *costus afer* had 11.6, 19.6 and 15.2% inhibition respectively. *Aspergilus niger* there was high inhibitory effect of 69.3, 71.1 and 64.9% respectively. Whereas at 30% of different extract of *costus afer*, had the highest inhibitory effect on *Aspergillus niger* which are 82.5, 89,5 and 85.9% respectively. The highest percentage inhibition of mycelial growth of *Aspergillus niger* were associated with 30% extract of *costus afer* and the lowest percentage inhibition were associated with 10% extracts of *costus afer*. Table 1.

Extracts of costus afer on *Aspergillus flavus* at 10%, 20%, and 30% all showed inhibition (P =0.95) table. The ethanol extracts as showed has highest inhibition on the mycelia growth of *Aspergillus flavus*, at all level but 30% had the highest inhibition. Raw extract has moderate effect with an inhibition percentage of 15.7, 50.0 and 87.0 respectively on the mycelia growth of *Aspergillus flavus*. The water on aqueous extract of *costus afer* had the least inhibitory effect on the mycelia grow 9.26, 45.5 and 84.3 respectively. The control did not inhibit the growth of the fungus. All the percentages of extracts concentration were effective on the mycelia growth of *Aspergillus flavus*.

Table 3: Phytochemical Characteristics of Aqueous, Ethanol and Crude Extract of *Costus afer* Stem

Phytochemical	Aqueous	Ethanol	Raw
Phenols	+ +	+	+ +
Alkaloid	+ +	-	+ +
Saponins	-	-	-
Flavonoid	+ +	+	+ +
Tannins	+	-	+ +
Quinones	+ +	+	+ +

Abundant = + +

Trace = +

Absent = -

Phytochemical characteristics of different extracts revealed presence of phenols, alkaloid, flavonoid, tannins and quinones. However, Ethanol extract tested negative for alkaloid and tannins and saponins tested negative in all extracts of *costus afar*.

DISCUSSIONS

Several reports by some authors have been presented on pathogens associated with yam tube root in Nigeria and this study confirmed that *Aspergillus niger* and *A. flavus* are pathogenic. The fungal species that colonized the yam tuber must have been present in the atmosphere in the form of spores.

The effect of aqueous, ethanol and raw extracts of *costus afer* at three different concentrations (10, 20, and 30%) were evaluated in order to develop cheap and simpler methods of controlling rot of yam tuber in the country. In this study, the result showed that all the extracts of the plant at all the concentrations were inhibitory on the test organisms in – vitro but ethanol extract was more efficient than their corresponding extracts of the plant. This was in line with the work by (Okigbo et. al, 2012, Okigbo and Odu-ukwe 2009).

This can be attributed to the fact that ethanol is an organic solvent and will dissolve organic compounds better than any other solvent. (Okigbo et.,al. 2012). As the concentration of the *costus afer* extracts increases, the level of inhibition on the mycelial growth of the fungi also increased. This finding is similar to Okigbo (et. al, 2007) and Suleiman et. al, 2013. The finding is also similar to the results obtained by Suleiman (2010) who stated a significant difference between mycelial growths values recorded on the various plants extracts concentrations.

The results indicated the presence of alkaloid and tannins in aqueous and raw extract Alkaloids are known to have antimicrobial, antifungal and anti-inflammatory effect (Okwu et. al., 2004).

However, the potency of any biocontrol agent should be its ability to totally inhibit mycelia growth and sporulation from the first day of inoculation of pathogen. This will enable such biocontrol agent to serve as a curative and preventive biofungicide.

CONCLUSIONS

The extract of *costus afer* has shown potentials as a biofungicide in the inhibition of some pathogens. Also, further research is needed to be carried out to determine the actual concentration of *costus afer* extract that will totally inhibit the

growth and sporulation of the pathogens and also know the actual active ingredients responsible for the inhibition. The extracts can be developed as safe and cheap alternative fungicide for the use in reducing and controlling the rot of yam tuber in Nigeria.

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