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Evaluation of Plant Extracts for Antifungal Activity Against S*clerotium rolfsii* Causing Cocoyam Cormel Rot in Storage

Eunice. O. Nwachukwu and Osuji J.O.

Department of Plant Science & Biotechnology, University of Port Harcourt. Port Harcourt, Nigeria.

Abstract: The fungitoxic effects of *Cassia alata* (Candle bush) and *Dennetia tripetala* (Pepper fruit*)* leaf extracts on *Sclerotium rolfsii* causing cocoyam cormel rot in storage have been evaluated. Treatment with leaf powders and water extracts of the test plants significantly reduced the radial growth of the pathogen *in vitro* and the spread of the rot disease *in vivo*. The leaf powders of the test plants were found to be more effective in inhibiting the growth of the rot causing fungus in culture and reducing rot development in cocoyam cormels than the water extracts of leaves. The leaf powders and water extracts were more effective as bioprotectors on cocoyam cormels.

Key words: Cassia alata, Cocoyam, *Dennetia tripetala,* Leaf extract, *Sclerotium rolfsii.*

INTRODUCTION

Cocoyam (*Xanthosoma sagittifolium* L.) provides alternative source of carbohydrate to argument yams and cassava in Nigeria. It is used in essentially the same way as yam. It can be eaten boiled, fried or pounded into fufu, although it is not considered as prestigious as yam. It can also be made into porridge or pottage, as well as chips and flour. Cocoyam flour has the added advantage that it is highly digestible and so is used for invalids and as an ingredient in baby foods. In Nigeria, cocoyam is grated, mixed with condiments and wrapped in leaves. It is steamed for about 30 minutes and served with sauce.

Cocoyam ranks third in importance after cassava and yam among the root and tuber crops cultivated and consumed in Nigeria. Currently, Nigeria is the world's leading producer of cocoyam, accounting for up to 3.7 million metric tones annually FAO[11]. Cultivars of two species, *Colocasia esculenta* (taro) and *Xanthosoma sagittifolium* (tannia) are generally grown for food.

Diseases and pests in farm and storage appear to pose serious problem in cocoyam production and utilization. In storage, serious losses due to rotting of the corms and cormels are a major factor affecting adversely the quantity and quality of cormels for consumption and planting. The recycling of planting material (corms/cormels) year by year results in the accumulation of pathogens in them and this translates to yield decline with time. The 11% drop in national production figures between 2000 and 2004 was due to microbial attack by S*clerotium rolfsii* in storage FAO[10,11].

Microbial rotting and decay in stored cocoyam has been delayed with varying degrees of success following pre-storage application of fungicides as dips and dusts Jackson,[13]. Nwankiti *et al*[16]. highlighted some protective fungicides that have been found to be effective in keeping down some rot diseases but remarked that those chemicals were expensive and not usually easily available at prices that most farmers in Nigeria could afford. There is also the problem of lack of expertise in the safe handling of the fungicides among most of the farmers. The use of bio pesticides of plant origin has been suggested by some workers as alternatives to chemical use in order to counter the potential hazards and pollution problems associated with the use of synthetic chemicals Amadioha and Obi,[4] and Amadioha,[3].

It has been reported that plants with fungicidal properties are very effective in inhibiting fungal growth *invivo* and *invitro* Kuhn and Hargreaves[15]. *C. alata* and *D. tripetala* are among the plants with such properties Khan *et al*.[14].

Cassia alata (family: Caesalpinaceae) is an erect tropical, annual herb with leathery compounded leaves and yellow flowers. The leaves, flowers and seeds have been reported to contain high levels of anthraquinones, crysophanic, napthoquonine, or hennotannic acids which have been demonstrated traditionally to treat ringworm, eczema, itching skin infections in humans and very effective inhibitors of mite infestations, bacterial and microbial diseases (Anderson *et al*.[5], Khan *et al*[14]. Adeboye *et al*[1]. and Ali-Emmanuel *et al.*,[2]. *Dennetia tripetala* (Family: Anonaceae) is a woody plant at least 3 metre high with simple leaves

Corresponding Author: Eunice. O. Nwachukwu, Department of Plant Science & Biotechnology, University of Port Harcourt. Port Harcourt, Nigeria.

and abundant edible fruits widely consumed in Southern Nigeria. It has been reported that the essential oil of the fruit contains nearly 80% Phenylnitroethane and phenolic acid, which are toxic to some insects and micro- organisms Iwuala *et al*[12]. and Ejechi *et al*[8]. The extracts from the test plants have variously been reported as possessing antimicrobial and insecticidal properties against wide range of microorganisms and insects. However, reports of their effects on control of rots caused by *S. rolfsii* are sparse. This study therefore, investigates the efficacy of leaf powder and water extracts of *C. alata* and *D tripetala* against *S. rolfsii* causing soft rot of cocoyam cormel in storage. Storage of fresh corms is important for distant marketing, to free farmland for new cropping, and to ensure the availability of seed cormels in the next planting season.

MATERIALS AND METHODS

Source of pathogen and cocoyam cormel: Rotted cocoyam cormels (*Xanthosoma sagittifolium*) were collected from market stalls in Aba main market in Abia state Nigeria in sterile polyethylene bags. The cormels were washed with tap water and later with distilled water and surface sterilized with ethanol (70%) and left to dry for 30 minutes in order to avoid contamination by microorganisms.

Pieces of the infected tissues were cut and plated on sterilized potato dextrose agar (PDA) in Petri dishes. Subcultures were made to obtain pure cultures of the rot-causing organism, which was identified as *Sclerotium rolfsii* with reference to Barnett and Hunter[7].

The pathogenicity test of the fungus was carried out by surface sterilizing a sound cocoyam cormel and making a hole on the cormel with a sterile cork borer (5mm dia.). A disc of the fungus culture (5mm dia.) was introduced into the hole and the tissues previously removed from the hole was replaced after about 2mm had been cut off to compensate for the thickness of the inoculum. The point of inoculation was sealed with Vaseline and the inoculated cormel was placed in a micro humidity chamber and incubated for 7 days at 28^0C. A re-isolation was made on PDA from the rotted portion of the inoculated cormel and the isolate was compared with the original culture of the fungus for confirmation as the rot-causing organism.

The sound (uninfected) cocoyam cormels (*X. sagittifolium*) used in course of this experiment were obtained from the open market stalls.

Preparation of powder from *C. alata* and *D. tripetala* leaves: *C. alata* leaves were obtained from a thick forest near Obigbo and Afam in Rivers state. The leaves of *D. tripetala* were also obtained from a thick forest near Emohua in Rivers state. To obtain powder from these plant leaves, they were sun-dried for 2 days and ground to pass through a 0.4mm screen, using a hand driven grinder. The powders were put in plastic containers with tightly fitted lids, which were kept in the laboratory before use. The powders were used, within one week of preparation.

Preparation of leaf extracts : Fresh leaves of *C. alata* and *D. tripetala* were washed under running tap water and then distilled water before they were weighed separately (100g) and ground to form a paste. One hundred ml of distilled water was added to each paste, stirred vigorously and left to stand for 1hr and then filtered using a filter paper to obtain water extract of the test plants.

***In vitro* tests with leaf powders and water leaf extracts:** To 18ml of sterile potato dextrose agar placed in Petri dishes were added 2.5g of each plant leaf powders. The solution was mixed and allowed to solidify. The media were inoculated separately at the center with 4mm culture discs of *S. rolfsii*. The same method was used for the water leaf extract except that 2.5ml of the leaf extracts were mixed separately with 18ml of sterile potato dextrose agar placed in Petri dishes. The control was treated with 2.5ml of distilled water in place of the leaf powder and water leaf extract.

Three replicates of five plates each were maintained for each treatment and inoculated plates were incubated for seven days at room temperature (28^0C). The diameter of the radial growth of the fungus was measured at the end of the incubation period and then used to determine the fungitoxicity level of the powders and extracts using the formula:

$$\text{Percentage growth inhibition (\%)} = \frac{dc - dt}{dc} \times \frac{100}{1}$$

Where dc = average diameter of fungal colony in control treatment dt = average diameter of fungal colony with powder or extract.

In vivo tests with leaf powders and water leaf extracts: Two methods were used to test for the efficacy of the powders and extracts *in vivo*. The first batch involved the robbing of leaf powders as a slurry and spraying of leaf extracts separately on the sound cocoyam cormels two days before spray inoculating with spore suspension (10×10^5 spores/ml distilled water) of *S. rolfsii*.

In the second batch, the healthy uninfected cormels were spray-inoculated with the spore suspension of the pathogen before the application of the leaf powders and leaf extracts 2 days later. The infected cormels were

placed in a micro incubator for 14 days at room temperature. Distilled water only was used in place of the powders and extracts of the test plants in the control experiment. The severity of the infection after the incubation period was measured on a 0 – 4 scale.

- 0-no infection
- 1-slight infection
- 2-moderate infection (50% of cormel infected.)
- 3-severe infection (75% of cormel infected)
- 4-complete rot (100% infection).

The experiment was repeated three times and mean values used. The data was analyzed with ANOVA and the means were separated using LSD at a probability level of 5%.

RESULTS AND DISCUSSIONS

The radial growth of *S. rolfsii* in culture was significantly suppressed by powder and water extract of leaves of *C. alata* and *D. tripetala* when compared with control (Table1.). Leaf powders of *C. alata* recorded highest growth inhibition followed by the leaf powder of *D. tripetala* though the results were not significantly different (P< 0.05). The leaf powders of the test plants were more effective than the water extracts.

Table1. Effect of powders and water extracts of *C.alata* and *D. tripetala* leaves on the radial growth of *Sclerotium rolfsii* in culture after 7 days of incubation (n=3).

Plant material	Radial growth (cm)	Leaf powder water extract	Growth inhibition (%)	Leaf powder water extract
C. alata	1.0	3.5	88.8 + 1.69[a]*	61.1 + 1.5[b]
D. tripetala	1.2	3.7	86.6 + 1.59[a]	58.8 + 0.8[b]
Control (water)	9.0	9.0	0	0

Values are means of three replicates in two separate experiments.

*a,b = values with different superscripts within the same column are significantly different (P < 0.05).

Table 2. Effect of leaf powders and water leaf extracts of *C. alata* and *D. tripetala* on rot development in cocoyam cormels caused by *S. rolfsii* after 14 days incubation (n=3).

Plant material	Disease severity			
	2 days before inoculation		2 days after inoculation	
	Leaf powder	water extract	Leaf powder	Water extract
C. alata	0	1	2	3
D. tripetala	1	2	2	3
Control (water)	4	4	4	4

This suggests that, water used in the extraction process was probably not able to dissolve all the active principles/compounds present in the leaves, which are contained in the leaf powder.

Table 2 shows the results of the effects of leaf powders and water leaf extracts on the spread of the disease in cocoyam cormel (*in vivo).* The leaf powder and water extract of the test plants suppressed the cormel rot of cocoyam when it was applied two days before inoculation with the fungus. This result indicates that the leaf powders and water leaf extracts of the test plants could be more effective as protective than curative substances.The cocoyam cormels treated with water before and after spray inoculating with the fungus rotted completely. The leaf powder of *C. alata* and *D. tripetala* gave better protection of the cocoyam cormels than the leaf extracts. However the leaf extracts were significantly better than the control.

This study reveals that *C. alata* and *D. tripetala* contain fungitoxic compounds since they were able to suppress the growth of *S. rolfsii invitro* and *invivo*. This agrees with earlier reports by some researchers on antimicrobial activities of properties from these plants and their insecticidal effects on mites Ali- Emmanuel, *et al*[2]. Khan *et al*[14]. Anyaele and Amusan,[6]. and Anderson *et al*[5]. Ejechi *et al*[8]. reported that the essential oil and phenolic acid extracts of *Dennetia tripetala* inhibited the growth of tomato-rot fungi. Ejechi *et al*[9] in another study, also reported that all isolated micro organisms (*Staphylococcus aureus, Salmonella* sp., *Pseudomonas aeruginosa. Proteus* sp., *Escherichia coli., Enterococcus faecalis., Serratia* sp., *Bacillus* sp., *Clostridium* sp., *Penicillium* sp., *Aspergillus flavus*) from food products were susceptible to *D. tripetala* extracts with a minimum inhibitory concentration (MIC) range of 1.0-4.0 mg/ml.

It is therefore recommended that the leaf powders of *C. alata* and *D. tripetala* should be used for the prevention of cormel rots of cocoyams caused by *S. rolfsii*. The use of the leaf powders of these plants as bioprotectants will constitute a modern tactic of cormel treatment for cocoyam disease control without hazards.

It should be noted that these plant extracts are of low cost and abundantly available. They require simple techniques to prepare and apply. In addition, they are very safe to handle than fungicides and leaves no residue on the cormels.

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