ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 2 Issue 12 December 2019

Research Article

Antifungal Activities and Phytochemical Analysis of Leaf Extracts of *Azadirachta Indica* and *Tridax Procumberns* on Fungi Associated with *Dioscorea Alata* (White Yam) Tuber Rot in Storage

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Received: October 28, 2019; **Published:** November 14, 2019

DOI: 10.31080/ASMI.2019.02.0439

Abstract

Yam (*Dioscorea alata*) is an important staple food in Nigeria. With the increase in population, there is a huge deficit between consumption and production levels of this crop. This situation is aggravated by post-harvest tuber rot fungal diseases. The present study was conducted to assess the efficacy of two plant extracts (*Azacdirachta indica* and *Tridax procumbens*) on fungi associated with this diseases in Mkpat Enin Local Government of Akwa Ibom State, Nigeria. A market survey of Ukam and Ekim markets indicated relatively high disease incidences of 35% and 42% respectively. Pathogenicity test confirmed five fungi (*Alternaria solanii, Fusarium oxysporum, Pestalotia guepini, Lasiodiplodia theobromae* and *Sclerotium rolfsii*) as causal agents of this disease. Results of phytochemical screening of two plant extracts tested showed high levels of secondary metabolites (alkaloids, saponins, tannins, terpernoids, flavonoids, glycocides and polyphenols). Data obtained from *in vivo* and *in vitro* tests of these extracts also, revealed high levels of pathogen growth inhibitions which ranged between 60% and 82%. *A. indica* and *T. procumbens* are therefore, recommended as alternative bio-pesticides that could replace highly toxic and environmentally unfriendly synthetic agro-chemicals.

Keywords: Bio-Pesticides; *Agro-Chemicals; Dioscorea Alata; Azacdirachta Indica; Tridax Procumbens;* Phytochemical Screening

Introduction

Yams (*Dioscorea* spp.) are among the most important staple foods in the world, especially some parts of the tropics and subtropics [1]. The role played by yam in the food economy in most West African countries cannot be over- emphasized. It is one of the most important dietary sources of energy produced within the tropics. Significantly, yam contributes to food security in Nigeria and other West African countries. The income generated by rural poor farmers who are engaged in yam production improves their living standards. Properly stored yam tubers represent stored wealth which can be sold all-year-round by the farmer or marketer. FAO [2] reported that Nigeria produces about 66.6% (26.6 million metric tons) of total world's yam production, with Ghana producing 9.8% (3.9 million metric tons) every year.

However, there are several constraints to the yam industry in the country. Of these constraints, diseases contribute greatly to high yield losses before and after harvest. Yam plants are prone to infection by fungi, bacteria, and viruses at all stages of growth and also during storage of tubers.

Tuber rot is a major factor limiting the post-harvest life of yams and losses can be very high. Losses due to post-harvest rot significantly affect farmers' and traders' income, food security and seed yams stored for planting. The quality of yam tubers is greatly affected by rots, which make them unpalatable to consumers. In Nigeria, over 60% of white yam varieties get rotten when stored for less than six months [3]. The control of tuber rot diseases of yams have been dominated by use of agro-chemical which are toxic and environmentally unfriendly. There is therefore, need to develop cost effective and less toxic control measures. The use of natural plant products is being tested this research.

Materials and Methods

Material

All the materials used in this study were acquired from Mkpat Enin Local Government Area in Akwa Ibom State, Nigeria, while the equipment, chemicals and glass wares were obtained form the Departments of Botany and Chemistry, Akwa Ibom State University Nigeria.

Methods

Survey /physical examination of samples

Two markets; Ukam and Ekim in Mkpat Enin Local Government Area, Nigeria were surveyed for yam tuber rot. A total of twenty white yam tuber samples were collected at random from each of the two markets. The collected samples were placed in separate sterile polythene bags and conveyed to the laboratory for further examination. Physical examination of naturally infected yam tubers was conducted using the method of Amusa., *et al* [4]. Tubers were considered infected when they show evidence of rot. Percentage infection was also assessed to determine the severity or incidence of rot in each of the two markets using the formula:

$$\text{Disease incidence \%} = \frac{\text{(No.of infected tubers)}}{\text{(Total No.of tubers examined) x100}}$$

Isolation and identification of fungal species

Fungal isolation was conducted following Okey [5] protocol. With the aid of a sterile cock borer, portions of yam tissues showing advancing margin of rot were obtained. These pieces were surface sterilized with 70% Ethanol for 5mins and rinsed with three changes of sterile distilled water. Each of these pieces were used in inoculating Potato Dextrose Agar (PDA) plates, with one piece per plate. The plates were incubated at 27 ± 2 °C for 7 days. Isolates were subculture to obtain pure cultures. Identification was based on morphological characteristics such as pigment production, colony texture, spores or conidia-etc.

Pathogenicity test

Pathogenicity test was conducted using a modified protocol of Okigbo and Ikediugu [6]. One week old pure cultures were used as source of inoculate for this test. Healthy yam tubers were surface sterilized with 5% Sodium hypochlorite and rinsed with sterile distil water. Using a 5mm diameter cork borer, holes were drilled on the tubers. These holes were inoculated with mycelial disk from young growing cultures and were then covered with Vaseline to prevent secondary contamination. Controls were set-up by inoculating tubers with sterile distil water. Experiments were replicated thrice and incubated at room temperature for seven days. Observations were made for the development of rot. The fungi were re-isolated and identified to confirm their identities.

Plant extract preparation

Dry leaves of *Azacdirachta indica* (neem) and *Tridax procumbens* were grounded manually and 20g of each was used for aqueous and ethanol extraction. The filtrates from aqueous extract was used for *in vivo* and *in vitro* tests while the ethanoic extract was used for phytochemical screening

Phytochemical analysis

Phytochemical tests were conducted following standard procedures to identify different constituents such as alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides and polyphenol [7].

In-vitro antifungal assay

The efficacy of the two extracts was tested by incorporation 10, 20 and 30% extracts into PDA on Petri dishes. Plates were also prepared with similar concentrations of a fungicide (Shavit wp). The plates were inoculated as previously stated and incubated at room temperature for 7days. Controls were arrangement without extracts. Experiments were setup in three replicates. Measurement of the mycelia radial growth was done daily for seven days [8]). Percentage inhibition was assessed using the formula:

$$\%\ \text{growth inhibition} = \frac{dc - dt}{dc} X \frac{100}{1}$$

Where: dc = diameter of colony in control experiment; dt = diameter of colony in treated plants

In-vivo antifungal assay

In-vivo antifungal assay of the plant extract was carried out using Okey [9] protocol. Healthy yam tubers were surfaced sterilized using 70% Ethanol, rinsed with distilled water and allowed to dry for 2 - 3 minutes. The sterile tubers were then sprayed with different concentrations of the extract (10, 20 and 30%) and separate tubers sprayed with similar concentrations of a fungicide (Shavit wp). The sprayed tubers were then inoculated with disk of fungal isolates as earlier described. Controls were setup with tubers inoculated with non-fungal disks. Growth rate was measured as previously stated for three weeks at two days intervals.

Results

Survey and physical examination

Survey results from Ukam and Ekim markets indicated high disease incidences of yam rot of 35% and 42% respectively. Infected yam tubers were brown in colour, with hard and dry regions as compared to healthy yam tubers (Plate 1 A, B and C).

Plate 1A: Yam tubers infected by Dry Rot; 1B: Soft Rot and 1C: Healthy yam tubers.

Identification of fungal isolates

Five fungi were isolated and based on cultural and microscopic characteristics they were identified as *Alternaria solanii, Fusarium oxysporum*, and *Pestalotia guepini* (Plates2 A, B and C) respectively. *Lasiodiplodia theobromae* and Sclerotium *rolfsii* were also identified accordingly. While the first three fungi were found in Ukam market, the last two were obtained form Ekim market.

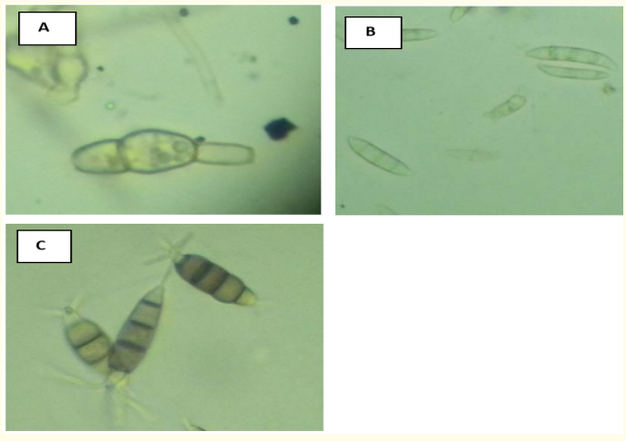

Plate 2: Conidia of A: *Alternaria solani*; B: *Fusarium oxysporum* and C: *Pestalotia guepini.*

Pathogenicity test

All the five isolates (*A. solanii, F. oxysporum, L. theobromae, P. guepini* and *S. rolfsii*) were found to be pathogenic on yam. While *A. solanii, L. theobromae* and *P. guepini* caused dry rot on yam, *S. rolfsii* and *F. oxysporum* caused wet rot on tubers.

Phytochemical analysis

Phytochemical screening showed the presence of seven secondary metabolites. Theses included; alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, and polyphenols (Table1). Extract of *A. indica* indicated relatively higher amounts of constituents compared to *T. procumbens.*

Constituents	Extracts	
	Azacdirachta indica	***Tridax procumbens***
Alkaloids	++	+
Saponins	+++	++
Tannins	++	+
Terpenoids	++	+
Flavonoids	+++	++
Ggycosides	++	+
Polyphenols	+++	++

Table 1: Phytochemical constituents in two extracts.

In vitro test

After 7 days of incubation and at 30% concentration of extracts and fungicides, results for two fungal pathogens showed significant levels of growth inhibition between extracts/fungicide and the control (Table2). However, inhibitory levels were not different between extracts and the fungicide tested. Inhibitory results of other fungal pathogens showed similar trends.

Treatment	Percentage inhibition	
	Lasiodiplodia theobromae	***Sclerotia rolfii***
Azadirachta indica	62.00	63.00
Tridax procumberns	60.00	62.00
Fungicide	63.00	65.00
Control (Water)	0.00	0.00

Table 2: Percentage inhibition of two fungal pathogens at 30% concentration after 7 days of incubation.

In vivo test

In vivo results of *L. theobromae* revealed significant difference in inhibition levels between the extracts/fungicide and the control (Figure 1). Growth inhibition was higher with extracts compared to the fungicide. The rate of inhibition decreased with time. In addition, the two extracts recorded significantly higher inhibitory levels compared to the tested fungicide (Figure 1). The other tested pathogens also showed similar trends.

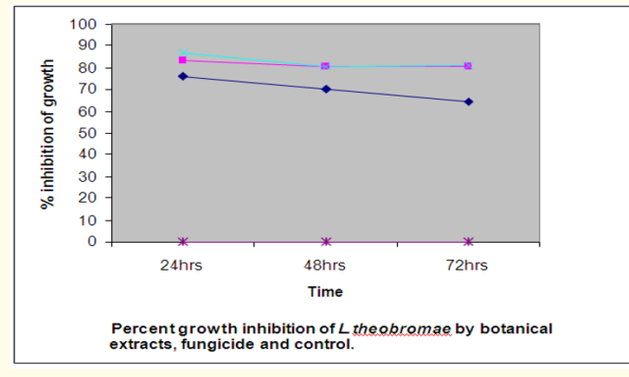

Figure 1: Percentage inhibition of *L. theobromae* at 30% concentration

Key:AI= Azadirachta indica (extract); TP = Tridax procumberns (extract); SH = Shavit wp (fungicide); CON = Control (water).

Discussion

Disease is the second most important problem after inadequate finances which affects yam cultivation worldwide [10]. Yam tuber rot diseases are associated with post-harvest handling. Although,

the present study revealed disease incidence as high as 42% this can not be compared to the 80% reported by Vinayaka., *et al.* [11]. It is therefore, not surprising that some level of attention have been paid to this disease.

From this study, five fungal pathogens were found to cause yam tuber rot in storage. These included: *Lasiodiplodia theobromae, Fusarium oxysporum, Alternaria solani, Pestalotia quepini,* and *Sclerotim rolfsii*. However, in addition to the above, a wider pathogenic range have been reported including: *Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae, Penicillium chrysogenum, Rhizoctonia spp., Penicillium oxalicum, Trichoderma viride,* and *Rhizopus nodosus* [12,13,14]. The implication the above fungal pathogens in a wide range of plant diseases is an indication of their high level of virulence.

Reports on the use of natural plant products as bio-pesticides for the control of post-harvest rot diseases of yam are scanty [13]. Bio-pesticides of plant origin are known to be more specific, biodegradable, cheaper, readily available and environmentally friendly when compared to synthetic chemicals. In this study the efficacies of two plant extracts (*Azadirachta indica* and *Tridax procumbens*) in controlling yam tuber rot fungi were significant. Phytochemical analysis of these extracts indicated high levels of secondary metabolites which have been reported as possessing antifungal properties [13,14]. Therefore, the inhibitory effects on the growth of causal agents observed in this study could be associated with these metabolites.

Conclusion

It should be noted that the inhibitory effects of the two plant extracts were significantly higher than the fungicide (Shavit wp) tested. This demonstrates the high efficacy of the extracts. *Azadirachta indica* and *Tridax procumbens* are therefore, recommended as bio-pesticides for the control of yam tuber rot in storage. Therefore, the two plant extracts tested could serve as alternatives to toxic fungicides. However, further studies are required to develop commercial quantities of these products.

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