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EVALUATION OF SOME BIO-PESTICIDES FOR THE CONTROL OF YAM TUBER ROT

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Abstract
Laboratory study was conducted to evaluate the efficacy of some botanicals on the control of yam fungal pathogens in Ebonyi State in 2017 cropping season. The laboratory study involved isolation and identification of fungi associated with rots in yam tubers. The test fungi isolates include: *Penicillium* spp, *Aspergillius* spp, *Botryodiplodia thebromae*, *Rhizopus stolonifer*, and *Trichoderma* spp were used for pathogenicity test on healthy yam tubers. The effects of plant extracts of garlic, ginger and moringa were used for the inhibition of growth of fungi isolates using pour plate technique. Data collected were subjected to one-way analysis of variance using the SPSS statistical package and mean separation carried out using DMRT at $p<0.05$. The main rot causal microorganism identified in stored yams was *Botryodiplodia theobromae*, which also had the least inhibition at 43.0% due to its virulence. Garlic extract showed the highest anti-microbial effect against all the test fungi, especially on *Trichoderma* spp. at 89.8% and *Panicillium* spp. at 83.9%, followed by ginger extract on *Penicillium* spp. at 68.7% and *Aspergillius* spp at 54.1%, moringa extract on *Penicillium* spp at 71.0% and *Aspergillius* spp at 55.9%. All the extracts at 100% concentration inhibited the five fungi isolates with *Penicilium* spp at 78.4%, *Aspergillius* spp. at 69.6%, *Botryodiplodia theobromae* at 37.5%, *Rhizopus stolonifer* at 40.5% and *Trichoderma* spp at 62.5%, which was significantly different from the results at 90% concentration. Thus, water based extraction technique was an effective method in inducing anti-fungal properties of garlic, ginger and moringa extracts. This research findings concludes that garlic, ginger and moringa extracts at 100% concentration possess anti-fungal properties to control fungal pathogens responsible for yam rots in Ebonyi State.

Keywords: Plant extracts, Rot, Fungi, and Yams

Introduction

Cultivated yams belong to the family *Dioscoreacea* and to the genus *Dioscorea* (Nzogbu 2014). The most cultivated species in Nigeria are the *D. rotundata* (white yam), *D. cayenesis* (yellow yam), and *D. alata* (water yam). Nigeria ranks first as the highest producer (47.53mmt) of yams in the world with about 65.49% contribution to total world production after Ghana (10.83%) and Cote d'ivoire (9.99%) (FAO, 2018).

Amusa (2003) estimated losses of up to 50% of fresh matter, with fungi as the primary casual agents of storage rots, which contribute to past harvest storage losses of yam. The basic pathogenic fungi of yam tuber includes: *Aspergillus flavus, Aspergillus niger, Aspergillus tamari, Botryodiplodia theobromae, Cladosporium herbarum, Fusarium oxysporum, Fusarium solani, Penicillium chrysogenom, Penicillium oxalicum, Rhizopus nodosus, Rhizctonia spp.* and *Trichoderma viride.* (Shiriki, 2015 and Okigbo and Nmeka, 2005).

Several methods have been developed for the storage of yams. However, the traditional yam barn, in spite of its inadequacies, remains the most popular among farmers. Therefore, post harvest storage losses remain an impediment in the production of this important crop. The control of post harvest diseases has been mainly based on synthetic fungicide application such as, thiabendazole, imazalil and sodium ortho-phenyl phonate (Harbant and Ghassan, 2011). These fungicides have major drawbacks including: high cost, unavailability to farmers, adverse effects on the environment, targeted organisms develops resistance and most important, its phyto-toxicity to man and other none targeted organisms (Amienyo, 2007 and Allum, 2014).

The development of resistance to known agricultural pesticides by fungal pathogens in recent years has become a very serious problem in crop production and protection. This phenomenon of resistance has been

reported to occur within 7 to 10 years post-introduction of every given agricultural chemical formulated to control pathogenic fungal organisms (Deshi *et al.*, 2014). Recently, the antimicrobial activity of some natural products such as plant extracts and essential oils that are biodegradable, safe to human health, cheap and readily available has attracted the attention of international and national researchers in the control of plant and post harvest diseases (Shiriki, 2015; Deshi *et al.,* 2014; Markson *et al*., 2012; Nweke, 2015), but the actual use of these products for the control of postharvest pathogens of tubers generally, and in particular for yam pathogens is however, still limited. The specific objectives of the study were to identify the fungi that causes yam tuber rots, investigate the efficacy of three bio-pesticides in managing and protecting the rot in yam tubers during and before storage.

Materials and Methods

Source of Yam Tubers and Plant Materials

Rot diseased white yam tubers (*Discorea rotundata*) and water yam (*Discorea alata)* were collected from the yam barns of yam farmers in Ebonyi state. These were packaged in polyethylene bags and taken to the laboratory of National Root Crops Research Institute, Umudike. Healthy yam tubers were also obtained from the yam barns of same respondents. The tubers were washed and rinsed in running tap water before being used for pathogenicity test. The plants: *Zingiber officinale* (rhizome), and *Moringa oleifera* (leaves) used in the experiment, were collected from the vegetable gardens of National Root Crops Research Institute, Umudike and *Allum sativum* (bulb) gotten from an open market all in Abia State. These plants were verified and authenticated in the Herbarium unit of Michael Okpara University of Agriculture, Umudike, Abia State.

Isolation and Identification of Fungi Associated with Rotted Yam Tubers

Rot diseased yam tubers were washed in tap water and cut into sections with sterilized scalpel. The sections were surface sterilized by placing in 70% ethanol for 2 minutes and rinsed with several changes of sterile distilled water. Four sections of the sterilized tubers were plated out on potato dextrose agar incorporated with streptomycin. The plated petri dishes were incubated at room temperature (28±2°C) for 7 days and observed daily for fungal development. The developing fungi were identified and pure cultures were prepared and stored in slants for further use (Okigbo and Nmeka, 2005; Barnet *et al.,* 1972; Amienyo, 2007 and Shiriki *et al.,* 2015).

Pathogenicity Test

The method of Shiriki (2015), was employed. Fresh healthy yam tubers were washed with sterile water for 10 minutes and surface disinfected with 70% ethanol for 2 minutes. They were rinsed by placing in three changes of sterile water for 3 minutes at each instance and dried on sterile plain sheets for 20 minutes in a laminar air flow cabinet. Six yam tuber lengths of *nvula* (water yam), and *ozibo* (white yam), were measured and divided into five segments for the five fungi isolates. These were done in triplicates. Cylindrical discs of the yams were removed aseptically with a sterile 7mm cork borer. A disc of 5mm of the organisms on the growth media was removed with a sterile 5mm cork borer and placed in each hole. This was covered by replacing the cylindrical disc and sealing with a vaseline. The inoculated tubers along side with the un-inoculated tubers (control) were placed in a safety chamber at room temperature (28±2°C) (Amienyo and Ataga, 2007), and incubated for 14 days. Then, tubers were transversely cut in each inoculated segment and examined for infection and disease development.

Preparation of Plant Extracts

The following plants: *Zingeber officinale* (rhizome)*, Allum sativum* (bulb)*,* and *Moringa oleifera* (leaves), were washed, dried and grounded separately. Ten grams of each sample were added to 10ml of distilled water in separate flasks. The samples were filtered with a sterilized cheesecloth and the filtrate used as the extract, kept in the refrigerator for antimicrobial activity test (Amienyo and Ataga, 2007; Shiriki, 2015).

Effect of the Extract on Fungal Growth

The method of Shiriki (2015), was used to determine the effect of the extracts on fungal growth. This involves creating a four equal section on each petri-dish by drawing two perpendicular lines at the bottom of the plate, the point of intersection indicating the centre of the plate. This was done before dispensing the potato dextrose agar (PDA) into each of the plates. About 10ml of the extract of the various plant materials were separately introduced into the petri-dish containing the media (PDA). A disc (5mm diameter) of the pure culture of the various fungi organisms were placed on the extract just at the point of intersection of the two lines drawn at the bottom of the petri-dish. Control experiments were set up without the addition of any plant material. Fungal toxicity was recorded in terms of percentage colony inhibition and estimated, following Okigbo and Nmeka (2005) thus;

$$\text{Percentage Growth Inhibition (\%)} = \frac{DC - DT}{DC} \times 100$$

Where;

DC = Average diameter of control, and

DT = Average diameter of fungal colony with treatment

Statistical Analysis

Descriptive statistics (percentages) were used to analyze the data collected on storage practices. Data collected from the laboratory experiment were subjected to analysis of variance (one-way ANOVA) by the use of the SPSS statistical package. Mean separation were carried out using the Duncan Multiple Range Test (DMRT) at $P < 0.05$.

Results and Discussion

Five storage fungi were isolated from the rot diseased yam tubers. The most frequent occurring fungi were *Penicillium* spp*., Aspergillius* spp*., Botryodiplodia*

theobromae, Rhizopus stolonifer, Trichoderma spp. (Table 1). The pathogenicity test revealed that these five fungi induced rot in healthy yam tubers of both *nvula* and *ozibo*. The most virulent of these fungi was *Botryodiplodia theobromae* in both *nvula* and *ozibo*. Water extracts of moringa, garlic (*A. sativum*) and ginger (*Z. officinale*) significantly (P=0.05) inhibited the radial growth of these fungi at different concentrations (Table 2), with *Penicillium* spp. having the highest rate of inhibition across all the bio-pesticides within the two concentrations. The control experiment showed an uninhibited growth of the pathogens. Garlic (*A. sativum*) extract showed highest inhibition across all the fungi organisms isolated both at 100% and at 90% concentrations (Table 3), followed by ginger (*Z. officinale*) and Moringa, with good inhibition at the undiluted concentration. *Penicillium* spp. was the most inhibited organism across the bio-pesticides. This is in agreement with earlier studies on the effects of these plants on phyto pathogens of other crops (Shiriki *et al.*, 2015 and 2019; Okigbo and Nmeka, 2010; Okigbo and Ikediugwu, 2000). Amienyo and Ataga (2007) used Z. *officinale* extracts to protect mechanically injured sweet potato tubers. Okigbo and Nmeka (2005), used extract of *Z. officinale* to control yam tuber rot. Aji and Tunwari, (2018), studied the antifungal effects of ginger rhizome extracts on the mycelial growth of some fungal pathogens of *Dioscorea rotundata*. In another study (Ijato, 2011), cold water and ethanol extracts of two fungicidal plants (*Zingiber officinale* and *Ocimum gratissimum*) screened for their *in vitro* effects on rot fungi of yam. In this study similar fungi were identified and found to cause rot to yam tubers. This study revealed that fungi-toxic compounds were present in Moringa, garlic (*A. sativum*) and ginger (*Z. officinale*), because they were able to inhibit the growth of fungi tested.

Conclusion

The study identified five different types of fungi disease causing micro-organisms in yam tubers which include; *Penicillium* spp., *Aspergillius* spp, *Botryodiplodia theobromae*, *Rhizopus stolonifer*, *Trichoderma* sp. This research findings therefore, have shown that the three bio-pesticides which include: garlic, ginger and moringa used have potency of managing pathogens responsible for yam rots at 100% concentration thereby prolonging shelf lives of yams.

Table 1: Percentage Occurrence of Micro Organisms Isolated From Rot Infected Yam

Isolates	Percentage Occurrence (%)
Penicillium sp.	7.0
Aspergillius sp	8.0
Botryodiplodia theobromae	50.0
Rhizopus stolonifer	12.0
Trichoderma sp	23.0

Table 2: Inhibition (%) of Radial Growth of Fungi in Potato Dextrose Medium Incorporated with Bio-Pesticides

Isolates	Garlic	Moringa	Ginger
Penicillium spp.	83.9a	71.0b	68.7b
Aspergillius sp	76.9a	55.9b	54.1b
Botryodiplodia theobromae	43.0a	11.4c	21.6b
Rhizopus stolonifer	55.7a	7.2b	15.8b
Trichoderma spp.	89.8a	21.3c	44.3b

Means with the same alphabets across the rows are not significantly different (P=0.05)

Table 3: Inhibition (%) of Radial Growth of Fungi in Potato Dextrose Medium Incorporated with Bio-pesticides of Moringa, Ginger and Garlic in Concentrated form of 100% and Diluted form of 90%

Isolates	100%	90%
Penicillium spp.	78.4a	70.7b
Aspergillius sp	69.6a	55.0b
Botryodiplodia theobromae	37.5a	13.2b
Rhizopus stolonifer	40.5a	12.0b
Trichoderma spp.	62.5a	41.0b

Means with the same alphabets across the rows are not significantly different (P=0.05)

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